2200/1637

TRANSMITTAL OF APPEAL BRIEF (Large Entity)

Docket No.
PU9993CIP2

In Re Application Of: George W. Hawkins

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/605,766	06/28/2000	Heather Calamita	22840	1637	5882

Invention: **Method and Apparatus for Performing Biological Reactions on a Substrate Surface**

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on **August 18, 2005.**

The fee for filing this Appeal Brief is: **$500.00**

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Royal N. Ronning, Jr.
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Dated: **October 14, 2005**

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appl. No.	:	09/605,766	Confirmation No.: 5882
Applicant	:	George W. Hawkins	
Filed	:	June 28, 2000	
TC/A.U.	:	1637	
Examiner	:	Heather Calamita	
Docket No.	:	PU9993CIP2	
Customer No.	:	22840	

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October 14, 2005

APPEAL BRIEF

Sir:

Appellant submits this Appeal Brief in triplicate, appealing from the May 16, 2005, rejection of the Primary Examiner, finally rejecting claims 1, 36, 38–60 and 64, in the captioned application. The Notice of Appeal was filed on August 18, 2005, which contained authorization to charge the "Appeal Fee" to Appellant's Deposit Account. Filed concurrently herewith is the "Transmittal of Appeal Brief (Large Entity)", in duplicate, which contains authorization to charge the fee for filing the Appeal Brief to Appellant's Deposit Account.

Real Party in Interest

Amersham Biosciences AB, the assignee and owner of the captioned application, is the real party in interest to this appeal.

Related Appeals and Interferences

There are no other appeals or interferences related to the instant appeal.

Status of Claims

Claims 1, 36, 38–60 and 64 are pending in the captioned application. These are the only pending claims subject to examination before the U.S. Patent and Trademark Office. A copy of these claims is appended hereto.

Status of Amendments

There are no outstanding amendments with regard to the captioned application.

Summary of Claimed Subject Matter

The instant invention provides an apparatus for performing biological reactions on a substrate layer having a multiplicity of oligonucleotide binding sites disposed thereon. The apparatus provides a hybridization chamber including a flexible layer attached to a biochip by an adhesive layer. The biochip includes a substrate having a first and second surface, wherein the first surface contains a multiplicity of biologically reactive sites disposed in an area bounded by an adhesive layer set down on the first substrate surface (see e.g., page 4, lines 14–18), and further includes a flexible layer to create a reaction volume (see e.g., page 4, lines 19–22).

Claim 1 is directed to the apparatus wherein the surface contains an array of biomolecular probes positioned on the first surface (see e.g., page 11, lines 4–7) and at

least a first port through the flexible layer (see e.g., page 5, lines 3–17) and further wherein the adhesive layer forms a watertight bond with the first surface of the substrate and the flexible layer (see e.g., page 4, line 20 to page 5, line 3). Dependent claim 36 recites the biomolecular probes as oligonucleotides (see e.g., page 11, lines 10–15), dependent claim 38 recites the geometry of the first port as extending into the reaction volume (see e.g., page 15, lines 15–17 and page 21, lines 6–15). Dependent claims 40–42 further recite the material, which comprises the substrate (see e.g., page 17, lines 12–16). Dependent claims 43–44 recite how the biomolecular are anchored to the first surface (see e.g., pages 11–13). Dependent claims 45–46 further recite the presence of a heating under the reaction volume (see e.g., page 6, lines 4–10).

Claim 47 recites a system which contains a plurality of reaction volumes (see e.g., figure 5). Dependent claims 48–56 recite the material that may comprise a flexible layer (see e.g., page 18, lines 9–19). Claim 57 recites the apparatus including a scanner (see e.g., pages 23–24)) and claims 58 and 59 recites a system further including a sample preparation chip (see e.g., page 26, lines 9–17). Dependent claim 60 recites the apparatus including a roller in contact with the flexible layer (see e.g., page 25, line 15 to page 26, line 8). Claim 64 further recites that the reaction volume includes a water soluble compound that is solid at room temperature and liquid at a higher temperature (see e.g., page 4, line 21 to page 5, line 2).

Grounds of Rejection to be Reviewed on Appeal

1. Whether claims 1, 36, 37, 40, 45, 47, 49 and 57 are properly rejected under 35 U.S.C. § 102(b) as being anticipated by Cottingham, et al. (WO 97/10056, 03/20/1997).

2. Whether claims 43 and 44 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of Rehman, et al. (Nucleic Acids Research, January 1999).

3. Whether claims 48, 50–56 and 60 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of Bjornson, et al. (WO 99/19717, 04/22/1999).

4. Whether claims 39, 41, 42, 46, 58 and 59 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view Besemer, et al. (USPN 5,945,334, 08/31/1999).

5. Whether claim 64 is properly rejected under 35 U.S.C. § 1 03(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of Besemer, et al. (USPN 5,945,334, 08/31/1999) in further view of Van Antwerp, et al. (USPN 5,786,439, 07/28/1998).

Argument

1. **Claims 1, 36, 37, 40, 45, 47, 49 and 57 are not properly rejected under 35 U.S.C. § 102(b) as being anticipated by Cottingham, et al. (WO 97/10056, 03/20/1997).**

The Examiner has rejected claims 1, 36, 37, 40, 45, 47, 49 and 57 are not properly rejected under 35 U.S.C. § 102(b) as "being anticipated by Cottingham, et al. (WO 97/10056, 03/20/1997)".

Specifically, the Examiner stated, "Cottingham et al. teach an apparatus for performing biological reactions (see whole doc. esp. abstract & figure 4, DNA amplification and probe assay device) comprising a substrate (see page 13 line 3-4 DNA card with bottom and top layer) and an array with biomolecular probes positioned on first surface (see page 10 lines 1-15 teaching an array arrangement of DNA amplification and assay reagents which includes primers and probes spotted on surface) and flexible layer affixed to first surface by an adhesive layer forming reaction volume (see page 13 lines 9 & 10 adhesive binding a plastic film) and port (see page 13 line 21 & last line air vent and sample port). The ports extend through flexible layer (see Figure 4 detail 28 & 26)."

The Examiner continued, "The apparatus taught by Cottingham et al. is apparently watertight, as a port is described for entry of the sample. Cottingham et al. also do not describe sample leakage indicating the sample chamber is watertight (see p. 5 second paragraph). They teach apparatus may further comprise measuring instrument and heated carrier (see figure 13 detail 80, 81 and page 21 first full paragraph)".

In response, Appellant respectfully asserted that the Examiner had mischaracterized the reference. Specifically, as stated in the cited portion of the Cottingham, et al. reference, "The DNA card 20 (the apparatus of Cottingham et al) is

made up of a bottom layer 40, a middle layer 42 and a top layer 44. The seals 36 of the sealing strip 32 are adhered to the upper surface 46 of the top layer 44. Each of the layers 40, 42 and 44 is preferably made of a plastic film having a thickness of approximately 0.015 inches, and the seals 36 are of a similar material and thickness. The layers 40, 42 and 44 (together with the seals 36) are held together by a pressure sensitive adhesive (not shown) that is typically about 0.001 inch thick" (see page 13, lines 3–10).

Thus, Appellant submitted that quite different from the instant invention where the substrate has an upper and a lower surface, the Cards of Cottingham, et al. are comprised of three separate plastic layers, which are held together by pressure sensitive adhesives between each layer. Such is neither disclosed nor even suggested in the instant invention.

Further, Appellant stated that the Cottingham, et al. reference does not teach or disclose an array of biomolecular probes positioned in the first surface of the substrate within a reaction volume (or sample chamber), but rather teaches a plurality of sample chambers contained on the Card, each sample chamber containing a single spot of reagent (see e.g., page 13, line 16).

Additionally, Appellant pointed out that contrary to the Examiner's assertion, Cottingham, et al. neither discloses nor even suggests that the chambers are watertight, inasmuch as it does not mention this property at all. Appellant respectfully asserted that for the Examiner to properly maintain an objection under 35 U.S.C. § 102(b), the features

must be positively recited, and not merely inferred from the fact that the Cottingham, et al. reference does not describe sample leakage.

In response to the first part of the argument, wherein Appellant pointed out that the cards of Cottingham, et al. comprise three separate plastic layers held together by pressure sensitive adhesives between each layer, the Examiner stated, "The claim[s] uses the open language of comprising and therefore it is irrelevant that Cottingham teach 3 layers". Further, in response to Appellant pointing out that Cottingham, et al. does not teach an array of biomolecular probes positioned in the first surface of the substrate, the Examiner stated, "Cottingham teach on p. 9 lines 7-8 and p 10 lines 1-2 a rectangular array of discrete sample cells which contain a dried DNA amplification reagent (nucleic acid primers, and a polymerase)". Further, in response to Appellant pointing out that the Cottingham, et al. reference does not teach that the chambers are watertight, the Examiner stated, "this feature was inherent as Cottingham discloses a sealed sample chamber. A sealed sample chamber is inherently watertight as the sample does not leak from the chamber."

In response, Appellant respectfully asserts that the Examiner's position cannot be sustained. The use of the open-ended term "comprising" is immaterial inasmuch as the apparatus claimed contains a substrate with a first and second surface, an adhesive layer, and a flexible layer adhered to said first surface by said adhesive layer. Cottingham, et al. provides no disclosure, or even any suggestion, of the middle layer and, indeed, the use of the open-ended language "comprising" does not change this. Indeed, the instant

claims specifically specify that the flexible layer is affixed to the first surface of the substrate by an adhesive layer. Such is neither disclosed nor even suggested by the Cottingham, et al. reference.

Further, Appellant respectfully submits that the Cottingham, et al. reference neither discloses nor even suggests "an array of biomolecules or probes positioned **on said first surface** within said reaction volume" (*emphasis added*), but rather teaches, as the Examiner correctly states, "a rectangular array of discrete sample cells which contain a dried DNA amplification reagent". These cells are described at pages 10–11 of the Cottingham, et al. reference, and are designed to be discrete cells that are separated from each other by sealing strips on the sides. Thus, unlike the instant invention, wherein "an array of biomolecular probes is positioned on said first surface within said reaction volume", in the Cottingham, et al. reference a series of separate reaction volumes is positioned on the first surface. Such is quite different from the instant invention, which is neither disclosed nor even suggested.

Additionally, Appellant again disputes the Examiner's assertion that a watertight chamber is inherent as Cottingham, et al. discloses a sealed sample chamber. Inasmuch as the word "sealed" is not defined, Appellant respectfully notes that the Cottingham, et al. reference does not disclose that the sample is watertight, and submits that it is improper to read such a property into the disclosure.

In view of the foregoing, Appellant respectfully submits that the Examiner's rejection cannot be sustained and should be reversed.

2. Claims 43 and 44 are not properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of Rehman, et al. (Nucleic Acids Research, January 1999).

The Examiner has rejected claim 43 and 44 under 35 U.S.C. § 103(a) as "being unpatentable over Cottingham et al. (WO 97/10056, 03/20/1997) in view of Rehman et al. (Nucleic Acids Research, January 1999)".

Specifically, the Examiner stated, "The teachings of Cottingham et al. are described previously. Cottingham et al. do not teach polyacrylamide. Rehman et al. teach polyacrylamide layer for binding probes (see p. 649, Introduction paragraph 2). One of ordinary skill in the art would have been motivated to apply polyacrylamide as taught by Rehmam et al. (Nucleic Acids Research, January 1999) polyacrylamide to the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) in order to immobilize DNA probes at a greater capacity".

The Examiner continued, "Rehmam et al. (Nucleic Acids Research, January 1999) state that polyacrylamide provides for great probe capacity, density, lower non-specific binding levels and relatively high thermal stability particularly in amplifications of solid phase PCR and hybridization assays (see p. 649, paragraph 2). It would have been prima facie obvious to apply the polyacrylamide as taught by Rehmam et al.

(Nucleic Acids Research, January 1999) to the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) for DNA probe assays in order to increase the hybridization efficiency of the probe reagents".

In response, Appellant reiterated the arguments as to the inapplicability of the Cottingham, et al. reference, and respectfully submitted that the addition of the Rehmam, et al. does nothing to remedy these deficiencies.

In response, the Examiner has stated, "Applicant's arguments with respect to these rejections have been considered but are moot in view of the clarification of the applicability of Cottingham's teachings. For all of the aforementioned reasons, the Examiner is not persuaded to withdraw the rejections against claims 1, 36, 38-60 and 64".

In response, Appellant respectfully reasserts the argument presented above as to the inapplicability off the Cottingham, et al. reference, and respectfully notes that the Examiner has cited Rehman, et al. for its teaching regarding polyacrylamide. Thus, Appellant respectfully submits that the references, alone or in combination with one another, neither anticipate nor render obvious the instant invention obvious.

In view of the foregoing, Appellant respectfully submits that the Examiner's rejection cannot be sustained and should be reversed.

3. Claims 48, 50–56 and 60 are not properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of Bjornson, et al. (WO 99/19717, 04/22/1999).

The Examiner has rejected claims 48, 50–56 and 60 under 35 U.S.C. § 103(a) as "being unpatentable over Cottingham et al. (WO 97/10056, 03/20/1997) in view of Bjornson et al. (WO 99/19717, 04/22/1999)".

Specifically, the Examiner stated, "The teachings of Cottingham et al. are described previously. Cottingham do not teach flexible layer with polyester, polypropylene. Bjornson et al, teach a variety of well known flexible films such ms plastics acrylics and polyethylenes of varying widths (see p. 17 line 15-17). Bjornson et al. teach rolling with roller (see figure 5). Bjornson et al. teach adhesives (see page 25 line 9)".

The Examiner further stated, "One of ordinary skill in the art would have been motivated to apply rollers and flexible films as taught by Bjornson et al. (WO 99/19717, 04/22/1999) to the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) in order to construct a cover for the reaction and press to ensure a seal of the film. It would have been prima facie obvious to apply rollers and flexible films as taught by Bjornson et al. (WO 99/19717, 04/22/1999) to the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) in order to ensure a sealed layer in Cottingham's device".

In response, Appellant reasserted the discussion presented above as to the deficiencies of the Cottingham, et al. reference and respectfully submitted that the addition of the Bjornson, et al. reference does nothing to remedy these deficiencies.

In response to this argument, the Examiner has stated, "Applicant's arguments with respect to these rejections have been considered but are moot in view of the clarification of the applicability of Cottingham's teachings. For all of the aforementioned reasons, the Examiner is not persuaded to withdraw the rejections against claims 1, 36, 38-60 and 64".

In response, Appellant reasserts the deficiencies of the Cottingham, et al. reference presented above and respectfully asserts that the addition of the Bjornson, et al. reference, which is cited by the Examiner for its teachings of rollers and flexible films, does not remedy these deficiencies. In summary, Appellant respectfully asserts that the references, alone or in combination with one another, neither anticipates nor renders obvious the instant invention.

In view of the foregoing, Appellant respectfully submits that the Examiner's rejection cannot be sustained and should be reversed.

4. Claims 39, 41, 42, 46, 58 and 59 are not properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view Besemer, et al. (USPN 5,945,334, 08/31/1999).

The Examiner has rejected claims 39, 41, 42, 46, 58 and 59 under 35 U.S.C. § 103(a) as "being unpatentable over Cottingham et al. (WO 97/10056, 03/20/1997) in view Besemer et al. (USPN 5,945,334, 08/31/1999)".

Specifically, the Examiner stated, "The teachings of Cottingham et al. are described previously. Cottingham et al. do not teach sample chip and heater Besemer et al. teach a chip device containing a substrate having an array of probes attached to cavity (see col. 1 line 65- col. 2 line 3 & claims 1 & 2). The body includes two inlets that allow fluids into and through cavity. A seal, plug or any other seal may be provided for each inlet to retain fluid within cavity (see col. 6 line 39). The body is formed by welding two pieces together. Besemer et al. also teach heaters may be connected to device (col. 9 line 62). Besemer et al. also teach of variety of surface supports including glass, silicon, Ge, GaAS (see col. 4 line 60-64)".

The Examiner continued, "One of ordinary skill in the art would have been motivated to use chips as taught by Besemer et al. (USPN 5,945,334, 08/31/1999) with the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) in order perform hybridization assays. Array chips were well known and commonly practiced in the art to perform detection assays. It would have been prima facie obvious to use chips as taught by Besemer et al. (USPN 5,945,334, 08/31/1999) with the device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) in order to perform a plurality of different assays simultaneously".

In response, Appellants respectfully reasserted the arguments as to the inapplicability of the teachings of Cottingham, et al and respectfully asserted that the addition of Besemer, et al. does not remedy these deficiencies.

In response to this argument, the Examiner has stated, "Applicant's arguments with respect to these rejections have been considered but are moot in view of the clarification of the applicability of Cottingham's teachings. For all of the aforementioned reasons, the Examiner is not persuaded to withdraw the rejections against claims 1, 36, 38-60 and 64".

In response, Appellant respectfully reasserts the deficiencies of the Cottingham, et al. reference described above, and respectfully assert that the addition of Besemer, et al., cited for its teaching regarding sample chips and heaters, does nothing to remedy these deficiencies. Accordingly, Appellant respectfully submits that the references, alone or in combination with one another, neither anticipates nor renders obvious the instant invention.

In view of the foregoing, Appellant respectfully submits that the Examiner's rejection cannot be sustained and should be reversed.

5. Claim 64 is not properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Cottingham, et al. (WO 97/10056, 03/20/1997) in view of

Besemer, et al. (USPN 5,945,334, 08/31/1999) in further view of Van Antwerp, et al. (USPN 5,786,439, 07/28/1998).

The Examiner has rejected claim 64 under 35 U.S.C. § 103(a) as "being unpatentable over Cottingham et al. (WO 97/10056, 03/20/1997) in view of Besemer et al. (USPN 5,945,334, 08/31/1999) in further view of Van Antwerp et al. (USPN 5,786,439, 07/28/1998)".

Specifically, the Examiner stated, "The teachings of Cottingham et al. and Besemer et al. are described previously. Cottingham et al. do not teach the claimed layer of water-soluble compound. Van Antwerp et al. teach coating the surface of biosensor with uniform hydrogel (see abstract). The hydrogel may be PEG 600 (see claim 10)".

The Examiner further stated, "One of ordinary skill in the art would have been motivated to apply PEG-600 coatings as taught by Van Antwerp et al. (USPN 5,786,439, 07/28/1998) to the chip array device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) and Besemer et al. in order to protect the array from interfering chemicals. Antwerp et al state that the hydrogel layer protects from interfering chemicals such as electrolytes and proteins but allows water to pass through to allow the arrays to accurately measure analyte (see column 1 lines 46-50). It would have been prima facie obvious to apply hydrogel as taught by Van Antwerp et al. (USPN 5,786,439, 07/28/1998) to the chip array device as taught by Cottingham et al. (WO 97/10056, 03/20/1997) and Besemer et al. in order to allow the array to accurately measure analytes without interference from other chemicals".

In response, Appellant reiterated the arguments above as to the inapplicability of Cottingham, et al. and Besemer, et al. and respectfully asserted that the addition of the Van Antwerp, et al. reference does nothing to remedy the deficiencies.

In response to this argument, the Examiner has stated, "Applicant's arguments with respect to these rejections have been considered but are moot in view of the clarification of the applicability of Cottingham's teachings. For all of the aforementioned reasons, the Examiner is not persuaded to withdraw the rejections against claims 1, 36, 38-60 and 64".

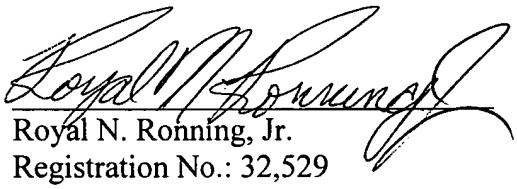
In response, Appellant respectfully reasserts the inapplicability of the Cottingham, et al. and Besemer, et al. references, alone or in combination with one another, as presented above, and respectfully submits that the addition of the Van Antwerp, et al. reference, which was cited for its teaching of coatings of the surface of biosensors of the uniform hydrogel, does nothing to remedy these deficiencies. Accordingly, Appellant respectfully asserts that the references, alone or in combination with one another, neither anticipates nor renders obvious the instant invention.

In view of the foregoing, Appellant respectfully submits that the Examiner's rejection cannot be sustained and should be reversed.

Conclusion

In view of the foregoing arguments, Appellant respectfully asserts that the Examiner's rejections cannot be sustained, and should be reversed.

Respectfully submitted,



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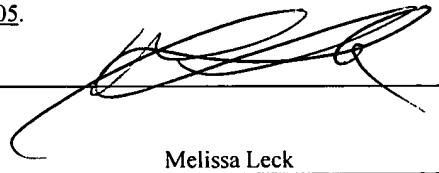
Signature: 

Name: Melissa Leck

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CLAIMS APPENDIX

The Rejected Claims

Claim 1 (previously presented): An apparatus for performing biological reactions comprising:

a) a substrate comprising a first and a second surface;

b) an array of biomolecular probes positioned on said first surface; and

c) a flexible layer affixed to said first surface by an adhesive layer, forming a reaction volume; and

d) at least a first port into said reaction volume; wherein said first port extends through said flexible layer.

Claims 2–35 (cancelled)

Claim 36 (previously presented): An apparatus according to claim 1 wherein said biomolecular probes are oligonucleotides.

Claim 37 (cancelled)

Claim 38 (previously presented): An apparatus according to claim 1 wherein said first port extends from said second surface to said reaction volume.

Claim 39 (previously presented): An apparatus according to claim 1 wherein said substrate comprises glass.

Claim 40 (previously presented): An apparatus according to claim 1 wherein said substrate comprises a polymer.

Claim 41 (previously presented): An apparatus according to claim 1 wherein said substrate comprises ceramic.

Claim 42 (previously presented): An apparatus according to claim 1 wherein said substrate comprises silicon.

Claim 43 (previously presented): An apparatus according to claim 1 wherein said biomolecular probes are anchored to said first surface using polyacrylamide.

Claim 44 (previously presented): An apparatus according to claim 1 wherein said biomolecular probes are anchored to a continuous layer of polyacrylamide.

Claim 45 (previously presented): An apparatus according to claim 1 further comprising a heating element positioned under said reaction volume.

Claim 46 (previously presented): An apparatus according to claim 45 wherein said heating element is a resistive heater.

Claim 47 (previously presented): An apparatus according to claim 1 comprising a plurality of arrays of biomolecular probes, and said flexible layer, said adhesive layer and said first surface comprise a plurality of reaction volumes each containing one of said arrays.

Claim 48 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises plastic.

Claim 49 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises translucent plastic.

Claim 50 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises rubber.

Claim 51 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises polyester.

Claim 52 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises Teflon.

Claim 53 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises polypropylene.

Claim 54 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises polyethylene.

Claim 55 (previously presented): An apparatus according to claim 1 wherein said flexible layer comprises polyvinylidene chloride.

Claim 56 (previously presented): An apparatus according to claim 1 wherein said flexible layer is a gas permeable membrane.

Claim 57 (previously presented): An apparatus according to claim 1 further comprising a scanner.

Claim 58 (previously presented): An apparatus according to claim 1 further comprising a sample preparation chip.

Claim 59 (previously presented): An apparatus according to claim 58 wherein said first port extends from said second surface to said reaction volume and said sample preparation chip is in contact with said second surface and wherein said sample preparation chip has a port that is aligned with said first port.

Claim 60 (previously presented): An apparatus according to claim 1 further comprising a roller, wherein said roller is in contact with said flexible layer.

Claims 61–63 (cancelled)

Claim 64 (previously presented): An apparatus according to claim 1 wherein said reaction volume further comprises a water-soluble compound that is a solid at room temperature and a liquid at a second, higher temperature.

EVIDENCE APPENDIX

Appellant hereby appends copies of the following:

1. U.S. Patent 5,945,334 to Besemer, et al.;
2. U.S. Patent 5,786,439 to Van Antwerp, et al.;
3. International patent application number PCT/US96/14681, which published as WO 97/10056;
4. International patent application number PCT/US98/21869, which published as WO 99/19717; and
5. Rehman, et al., (1999, January). Immobilization of acrylamide-modified oligonucleotides by co-polymerization. *Nucleic Acids Research, 27* (2), 649–655.

This is the evidence relied upon by the Examiner for rejection of appealed claims.

RELATED PROCEEDINGS APPENDIX

There are no other appeals or interferences related to the instant appeal.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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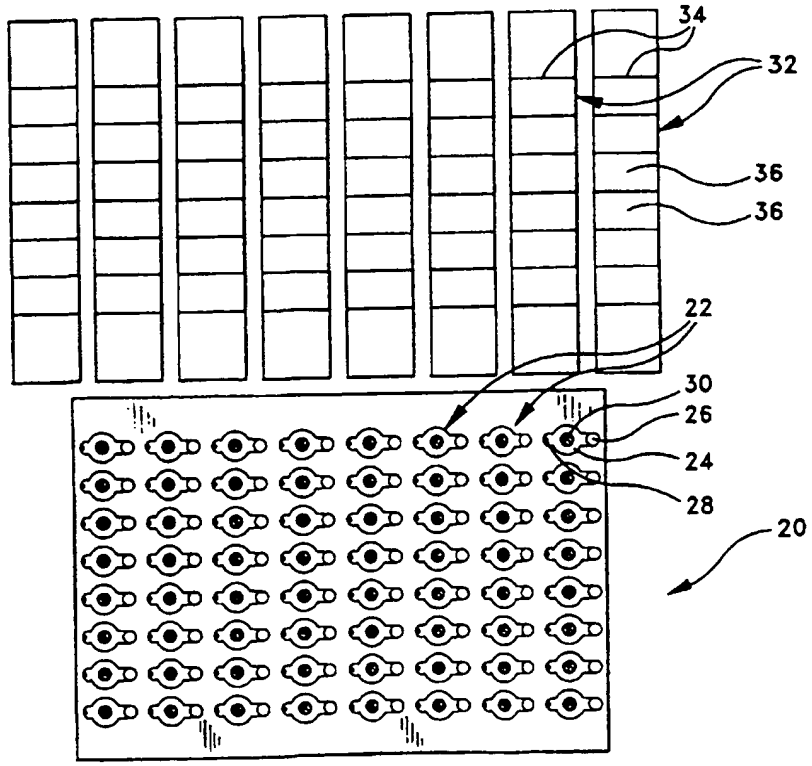
(54) Title: DEVICE AND METHOD FOR DNA AMPLIFICATION AND ASSAY

(57) Abstract

A DNA amplification and homogeneous DNA probe assay device is provided which includes a multiplicity of discrete sample cells in a flat "card" format, with each sample cell containing the reagents necessary for both DNA amplification and homogeneous DNA probe assay. The device is particularly suitable for fluorescence polarization DNA probe assays, and is preferably provided with an integral polarizer to avoid the need for polarizing elements in the related measuring apparatus. The size and geometry of the sampe cells allows for a "hot start" of the DNA amplification reaction and thereby avoids mispriming of the amplification reaction.



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DEVICE AND METHOD FOR DNA AMPLIFICATION AND ASSAY

Field of the Invention

The present invention relates generally to devices and methods for carrying out biological processes on liquid biological samples, and is particularly concerned with a unitary DNA amplification and homogenous DNA probe assay device which is suited for fluorescence polarization assays and is capable of accommodating a plurality of liquid biological samples in discrete, sealed sample cells.

Background of the Invention

The processes of nucleic acid (DNA) amplification and subsequent nucleic acid probe assay are well known and have been implemented in a variety of formats. While these formats are highly effective, they are somewhat difficult to perform in the clinical laboratory. Generally, DNA amplification and assay reactions are performed sequentially on the sample to be assayed; that is, the DNA amplification reaction is first carried out to completion, and the DNA probe assay is then performed on the fully amplified sample. This is referred to as an end point assay.

One problem with end point assays is that the amplified DNA (amplicons) from the DNA amplification reaction must be physically transferred to the subsequent DNA probe assay. Because of the transfer, the potential exists for contaminating the laboratory environment with the DNA amplicons. In addition, the general risk of misidentifying a given sample or confusing it with other samples increases each time that a physical transfer of the sample takes place.

There have been previous proposals for self-contained test units that are capable of carrying out an integrated nucleic acid amplification and nucleic acid assay on a liquid biological sample while the sample remains confined within the test unit. For example, U.S. Patent No. 5,229,297, to Paul N. Schnipelsky et al,

describes a cuvette for DNA amplification and detection which comprises a plurality of flexible compartments for containing a sample, amplifying reagents and detection reagents, together with passageways connecting the sample and reagent compartments with a detection site and waste compartment. A roller is used to squeeze or compress the sample and reagent compartments in a desired sequence, thereby forcing the sample and detection reagents through the passageways to the detection site and waste compartment. Temporary seals are used to isolate the sample and reagent compartments from the passageways until sufficient pressure is generated by the roller. Although this arrangement is advantageous in that the sample remains within the cuvette during amplification and detection, the need for a roller to break the temporary seals and cause the various fluids to flow between compartments introduces undesirable complexity and makes it difficult to automate the amplification and assay procedure.

In copending U.S. Patent Application Serial No. 08/277,553, filed by Hugh V. Cottingham on July 19, 1994, an improved test unit for carrying out integrated nucleic acid amplifications and nucleic acid assays is disclosed. In the improved test unit, the flow of sample and reagent liquids is controlled by centrifugal force applied by a relatively simple rotating apparatus, thereby avoiding the need for rollers and other complex mechanisms. While this represents a substantial improvement over the arrangement disclosed in U.S. Patent No. 5,229,297, the need to provide for controlled fluid movement within the test unit still exists and renders the test unit somewhat more complex than might be desired.

In addition to the end point assays discussed previously, homogenous methods of nucleic acid assay also exist. Homogeneous methods do not require the physical transfer of the amplified material to a separate assay site, but rather function simultaneously with the amplification reaction. Examples of known homogenous assay methods include fluorescence polarization, fluorescence energy transfer and light absorbance. While fluorescence polarization, in particular, functions very well in a research laboratory, it has a significant drawback in that

it requires glass sample tubes or cells. This is a result of the fact that most plastic processing methods, such as injection molding or thermoforming, create stresses in the material of the finished part. These stresses have random polarization effects, and interfere with the transmission of polarized light that is required for a fluorescence polarization assay.

As is well known, DNA amplification reactions must occur within a certain temperature range in order to produce the desired number of amplicons. If the sample and the DNA amplification reagents are allowed to react before the sample reaches the required temperature, a phenomenon known as "mis-priming" can occur. This can affect the validity of the assay results, both in the case of an end point assay and a homogeneous assay.

In view of the foregoing, a need exists for a device or a test unit which is capable of carrying out an integrated DNA amplification and DNA probe assay with minimal complexity, and preferably without requiring fluid movements to occur within the test unit itself. There is also a need for a test unit which can be used to carry out a homogenous DNA probe assay using fluorescence polarization methods, but which does not require the use of glass to properly transmit polarized light. Finally, there exists a need for a test unit which can be used to carry out an integrated DNA amplification and DNA probe assay in a simple and effective manner, while preventing inadvertent mis-priming of the amplification reaction. The present invention is directed to fulfilling these objectives.

It is an object of the present invention to provide a DNA amplification and homogenous DNA probe assay device in a "card" format that can be conveniently handled by clinical laboratory personnel, and accommodated in a suitable test apparatus.

It is another object of the invention to provide a unitary DNA amplification and DNA probe assay device which includes a multiplicity of sample cells, with each sample cell comprising the elements and reagents needed for a DNA amplification reaction and a homogeneous DNA probe assay.

It is a further object of the invention to provide a unitary DNA amplification and DNA probe assay device in which all reagents needed for both DNA amplification and DNA probe assay are contained, in dried form, within the device, so that the addition of a liquid biological sample is all that is needed to carry out the amplification and assay procedure.

It is a further object of the invention to provide a test unit and method that performs a "hot start" of the DNA amplification reaction, thereby avoiding an invalid assay result due to mis-priming of the amplification reaction.

It is a further object of the invention to provide a test unit which has the optical properties necessary for a fluorescence polarization assay, but which can be made of inexpensive plastic materials rather than glass.

It is a further object of the invention to provide a test unit and method that yields instantaneous DNA probe assay data by means of a kinetic or dynamic measurement of DNA amplicons, rather than a conventional end point measurement.

It is a further object of the invention to provide a fluorescence polarization DNA probe assay device which includes an integral polarizer, allowing for the use of a confocal polarization method.

It is a further object of the invention to provide an integral DNA amplification and homogenous DNA probe assay device that can be permanently sealed after the introduction of a liquid biological sample, thereby preventing amplicon contamination of the laboratory environment.

It is a still further object of the invention to provide an integrated DNA amplification and DNA probe assay device which can accommodate a plurality of liquid biological samples in discrete sample cells, and which can provide DNA probe assay data in a matter of minutes.

Summary of the Invention

In accordance with a preferred embodiment of the present invention, the disadvantages and limitations of the prior art are substantially avoided by providing a DNA amplification and homogeneous DNA probe assay device which includes a multiplicity of discrete sample cells in a flat "card" format, with each sample cell containing the reagents necessary for both DNA amplification and homogeneous DNA probe assay. The device is particularly suitable for fluorescence polarization DNA probe assays, and is preferably provided with an integral polarizer to avoid the need for polarizing elements in the related measuring apparatus. The size and geometry of the sample cells allows for a "hot start" of the DNA amplification reaction and thereby avoids inadvertent mis-priming of the amplification reaction.

In one aspect, the present invention is directed to an apparatus for carrying out a nucleic acid amplification and a homogeneous nucleic acid assay on a liquid biological sample. The apparatus includes a sample cell for receiving the liquid biological sample. The sample cell includes a sample chamber and a sample port for admitting the liquid biological sample into the sample chamber. A dried nucleic acid amplification reagent and a dried homogeneous nucleic acid assay reagent are adhered to the interior of the sample chamber for reacting with the liquid biological sample. A sealing member may be attached to the sample cell for sealing the sample port after the liquid biological sample has been admitted to the sample chamber. The homogeneous nucleic acid assay reagent may comprise a fluorescence polarization assay reagent, and in that event a portion of the sample cell may be made transparent to permit external detection of the fluorescence polarization reaction in the liquid biological sample. The sealing member may be attachable over the transparent portion of the sample chamber and may be made of a transparent, light-polarizing material to avoid the need for polarizing elements in the related measuring apparatus.

In another aspect, the present invention is directed to an apparatus for carrying out a biological process on a liquid biological sample. The apparatus comprises a substantially flat, card-like member having at least one sample cell therein for receiving the liquid biological sample. The sample cell includes a sample chamber, a sample port for admitting the liquid biological sample into the sample chamber, an air vent for allowing air to be displaced from the sample chamber during admission of the liquid biological sample into the sample chamber, and a dried reagent adhered to an internal surface of the sample chamber for reacting with the liquid biological sample. A sealing member, preferably in the form of a layer of flexible material carrying a pressure-sensitive adhesive, is attachable to the card-like member for sealing the sample port and the air vent after a liquid biological sample has been admitted to the sample chamber.

In another aspect, the present invention is directed to an apparatus for carrying out a nucleic acid fluorescence polarization assay on a liquid biological sample. The apparatus includes a sample cell for receiving a liquid biological sample. The sample cell has a sample chamber and a sample port for admitting the liquid biological sample into the sample chamber. The apparatus also comprises a dried nucleic acid fluorescence polarization reagent that is adhered to an internal surface of the sample chamber for reacting with the liquid biological sample. At least a portion of the sample cell is made of a light-transmissive, light-polarizing material to facilitate external detection of the fluorescence polarization reaction in the liquid biological sample without the need for separate polarization elements in the related measuring apparatus.

In a further aspect, the present invention is directed to a method for carrying out an integrated nucleic acid amplification and homogeneous nucleic acid fluorescence polarization assay on a liquid biological sample. The method comprises the steps of introducing a liquid biological sample into a sample well having a light-transmissive portion; bringing the liquid biological sample into

contact with a dried nucleic acid amplification reagent and a dried homogeneous nucleic acid fluorescence polarization assay reagent within the sample cell; sealing the sample cell; incubating the sample cell to allow the liquid biological sample to react with the nucleic acid amplification reagent and with the homogeneous nucleic acid fluorescence polarization assay reagent; and detecting fluorescence polarization in the liquid biological sample through the light-transmissive portion of the sample cell. The detection step may comprise directing polarized light through the light-transmissive portion of the sample cell or, if the light-transmissive portion of the sample cell is made of a light-polarizing material, directing unpolarized light through the light-transmissive portion of the sample cell.

In a still further aspect, the present invention is directed to a method for carrying out a nucleic acid amplification reaction on a liquid biological sample. The method comprises the steps of preheating a sample cell containing an dried nucleic acid amplification reagent to a temperature suitable for nucleic acid amplification; introducing a liquid biological sample into the preheated sample cell to bring the liquid biological sample into contact with the dried nucleic acid amplification reagent; equilibrating the temperature of the liquid biological sample to the temperature of the preheated sample cell; and, after the equilibration is substantially complete, commencing the nucleic acid amplification reaction in the sample cell. Preferably, the step of equilibrating the temperature of the liquid biological sample to the temperature of the preheated sample cell comprises forming a thin layer of the liquid biological sample in the sample cell to enhance heat transfer between the sample cell and the liquid biological sample.

Brief Description of the Drawings

The various objects, advantages and novel features of the invention will be more readily appreciated from the following detailed description when read in conjunction with the appended drawing figures, in which:

Fig. 1 is a top view of a DNA amplification and homogeneous DNA probe assay card constructed in accordance with a preferred embodiment of the present invention, illustrating separate sealing strips which are used to seal the individual sample cells of the card after liquid biological samples have been introduced into the sample cells;

Fig. 2 is a side view of the DNA amplification and DNA probe assay card of Fig. 1, illustrating its relatively small thickness;

Fig. 3 is a top view of the DNA amplification and DNA probe assay card and sealing strips of Fig. 1, with some of the sample cells shown filled with liquid biological samples and sealed using the sealing strips;

Fig. 4 is an enlarged cross-sectional view of a portion of the DNA amplification and DNA probe assay card of Figs. 1 - 3, with some of the discrete sample cells shown filled and others left empty to illustrate the locations of the dried amplification and assay reagents;

Fig. 5 is a plan view of the top layer of the DNA amplification and DNA probe assay card of Figs. 1 - 4, illustrating the manner in which sample ports and air vents are provided for the discrete sample cells;

Fig. 6 is a plan view of the middle layer of the DNA amplification and DNA probe assay card of Figs. 1 - 4, illustrating the keyhole-shaped apertures which define the side walls of the discrete sample cells;

Fig. 7 is a plan view of the bottom layer of the DNA amplification and DNA probe assay card of Figs. 1 - 4;

Fig. 8 is an exploded perspective view illustrating the relationship of the top, middle and bottom layers of the DNA amplification and DNA probe assay card of Figs. 1 - 4;

Figs. 9A and 9B are top view of two different embodiments of the sealing strips that are used to seal the sample ports and air vents of the DNA amplification and DNA probe assay card of Figs. 1 - 4;

Fig. 10 is an enlarged sectional view through one of the discrete sample cells in the DNA amplification and DNA probe assay card of Figs 1 - 4, illustrating the manner in which the use of a light-polarizing material for the sealing strips allows a confocal detection method to be used;

Figs. 11 and 12 are graphs depicting the change in fluorescence intensity with time during a DNA amplification and homogeneous DNA fluorescence polarization assay; and

Figs. 13 - 15 illustrate an exemplary measuring apparatus which can be used to measure fluorescence intensity in the sample cells of the DNA amplification and DNA probe assay card of Figs. 1 - 4.

Throughout the drawings, like reference numerals will be understood to refer to like parts and components.

Detailed Description of the Preferred Embodiments

A DNA amplification and homogeneous DNA probe assay device 20 (hereinafter referred to as a "DNA card") constructed in accordance with a preferred embodiment of the present invention is illustrated in Fig. 1. Although the specific dimensions and geometry of the DNA card may be varied in accordance with the requirements of particular applications, the card 20 of the preferred embodiment is rectangular with a length of approximately 5.025 inches and a width of approximately 3.362 inches. The card contains a rectangular array of discrete sample cells 22, spaced evenly across the length and width of the card. Each sample cell 22 includes a closed sample chamber 24 (the top wall of which is transparent) for receiving a liquid biological sample, an open sample port 26 which communicates with the sample chamber 24, and an air vent 28 which also

communicates with the sample chamber 24. Dried DNA amplification and assay reagents are adhered to the upper interior wall of each sample cell 22 in the form of a single, discrete spot 30. The sample ports 26 provides the means by which liquid biological samples (not shown in Fig. 1) can be introduced into each of the sample chambers 24 (preferably by pipetting), and the air vents 28 allows air to be displaced from the sample chambers 24 as the liquid biological samples are being introduced. As will be described hereinafter, the liquid biological sample that is introduced into the sample chamber 24 of each sample cell 22 makes contact with, and dissolves, the dried reagent spot 30 in the sample cell, thereby initiating the desired DNA amplification and assay reactions. Measurement of the assay results takes place while the liquid biological samples remain sealed within the sample cells 22, also in a manner to be described hereinafter. In the illustrated embodiment, the DNA card 20 contains sixty-four (64) identical sample cells 22, arranged in a rectangular array of eight rows by eight columns, on vertical centers of approximately 0.354 inches and horizontal centers of approximately 0.628 inches.

With continued reference to Fig. 1, sealing strips 32 (one for each column of sample cells 22 in the card 20) are provided for sealing the sample ports 26 and air vents 28 of the sample chambers 24 after liquid biological samples have been introduced into the sample cells 22. Preferably, the strips 32 are segmented along score lines 34 to define segments which can be used individually, if desired, to seal some of the sample cells 22 and not others. In the illustrated embodiment, the sealing strips 32 are approximately 0.628 inches in width and approximately 3.362 inches in length. Each sealing strip 32 seals a column of eight sample cells 22, with eight strips 32 being used to seal all sixty-four sample cells 22. The individual segments or seals 36 of each sealing strip 32 are on the same vertical centers (i.e., approximately 0.354 inches apart) as the sample cells 22 themselves. The sealing strips 32 are preferably about 0.015 inches thick and are provided with a layer of pressure sensitive adhesive on their lower surfaces

(not visible in Fig. 1). In practice, the sealing strips 32 are applied in a manner similar to adhesive tape, and serve to permanently seal the sample cells 22 by covering the sample ports 26 and air vents 28. The sealing strips 32 are generally used whole for convenience, and are only subdivided as necessary along the score lines 34.

The sealing of the sample cells 22 by means of the sealing strips 32 provides several advantages. First, the sealing strips 32 prevent evaporation of the liquid biological samples from the sample cells 22 during the DNA amplification and homogeneous DNA probe assay. Given that the volume of the liquid biological sample will typically be very small (about 20 μL) and that the amplification and assay reactions will usually take place at an elevated temperature (up to about 75° C), such evaporation may otherwise result in significant loss of the liquid biological sample. The second advantage of the sealing strips 32 is that they prevent the release of DNA amplicons from the sample cells 22, thereby preventing contamination of the laboratory environment. Finally, in accordance with a particularly preferred embodiment of the present invention, the sealing strips 32 may be made of a transparent, light-polarizing material so as to serve as polarization elements during the detection or measurement step. This avoids the need to provide separate polarization elements in the related measuring apparatus.

In the description which follows, it will be assumed that all of the sample cells 22 and seals 36 are identical, and that the description of any one sample cell 22 or seal 36 will apply to all. Although this is true in the preferred embodiment, the invention should not be regarded as being limited to this arrangement. It is within the scope of the invention to provide sample cells 22 and/or seals 36 that are different from one to the next, including (but not limited to) different reagents, different dimensions, different volumes and different optical properties. The reagents may differ for either the DNA amplification or the DNA probe assay, or both, with exemplary homogeneous DNA probe assay methods including

fluorescence polarization reactions, fluorescence energy transfer reactions and light absorbance reactions. Any or all of the foregoing differences may exist within a single card 20 and/or from one card 20 to the next. Thus, for example, different types of DNA cards 20 could be provided for carrying out different types of assays, with such cards retaining only a generic or functional similarity (e.g., to the extent necessary to fit into the same type of measuring instrument).

Fig. 2 is a side or edge-on view of the DNA card 20, which in the preferred embodiment has a generally flat or planar configuration. As will be apparent from Fig. 2, the DNA card 20 can be made extremely thin if desired. In the preferred embodiment, the thickness of the DNA card 20 is approximately 0.047 inch.

Fig 3. illustrates the DNA card 20 as it might appear during actual use, with fourteen of the sample cells 22 filled with liquid biological samples 38. In these filled sample cells 22, the liquid biological samples 38 have dissolved the dried reagent spots 30 of Fig. 1. The filled sample cells 22 are covered by the respective segments or seals 36 of the sealing strips 32. One complete sealing strip 32 has been applied to the left-hand column of sample cells 22, and a second sealing strip 32 has been subdivided along one of the score lines 34 into a first portion 32A which has been applied to the upper six sample cells 22 of the second column, and a second portion 33B which has been retained for future use. The unused portion 32B can be used to seal the two lowermost sample cells 22 of the second column during a subsequent use of the DNA card 20.

Typically, the various liquid biological samples 38 shown in Fig. 3 will consist of blood samples or other body fluid samples from different patients, all of which are being tested for the same pathogen by identical amplification and assay reagents 30. However, it will be understood that embodiments are possible in which more than one of the liquid biological samples 38 are drawn from the same patient, and in which the reagents 30 differ from one sample cell 22 to the next.

Fig. 4 is a partial cross-sectional view of the DNA card 20, taken along the line 4-4 in Fig. 3. In this view, the laminated construction of the DNA card 20 in the preferred embodiment can be readily appreciated. The DNA card 20 is made up of a bottom layer 40, a middle layer 42 and a top layer 44. The seals 36 of the sealing strip 32 are adhered to the upper surface 46 of the top layer 44. Each of the layers 40, 42 and 44 is preferably made of a plastic film having a thickness of approximately 0.015 inches, and the seals 36 are of a similar material and thickness. The layers 40, 42 and 44 (together with the seals 36) are held together by a pressure-sensitive adhesive (not shown) that is typically about 0.001 inch thick. Referring for convenience to the empty sample cell 22A in Fig. 4, the sample port 26 is preferably about 0.125 inch in diameter and communicates with a narrow section 48 of the sample chamber 24 that is preferably about 0.125 inch wide. The narrow section 48, in turn, communicates with a larger, substantially circular portion of the sample chamber 24 which is approximately 0.250 inch in diameter. The dried reagent spot 30 is adhered to the upper wall of the circular portion of the sample cell 24 (corresponding to the lower surface of the top layer 44 of the DNA card) and is situated approximately at the center of this circular portion. The circular portion of the sample chamber 24 communicates with another narrow section 52 of the sample chamber which is approximately 0.125 inch in diameter. The section 52, in turn, communicates with the air vent 28 located at the opposite end of the sample cell 22A from the sample port 26. The air vent 28 is preferably about 0.040 inch in diameter. The height of the interior of the sample chamber 24 is defined by the thickness of the middle layer 42 of the DNA card 20, and by the thickness of the adhesive on either side of this layer. This results in an overall height of about 0.017 inch for the interior of the sample chamber 24.

With continued reference to Fig. 4, the sample cell 22B is shown as it would appear during use. Thus, the sample cell 22B is filled with a liquid biological sample 38 and sealed with a seal 36 which covers the sample port 26

and air vent 28. The dried reagent spot 30 of Fig. 1 has been dissolved by the liquid biological sample 38.

In order to use the DNA card 20, a suitable measuring instrument is required. Depending upon whether the DNA card 20 contains assay reagents for fluorescence polarization reactions, fluorescence energy transfer reactions or light absorbance reactions, and whether (in the case of a fluorescence polarization assay) the DNA card 20 has integral polarizing elements, the instrument may be either a conventional instrument, such as a microplate fluorometer or a microplate reader, or a specialized instrument of the type to be described shortly in connection Figs. 13 - 15. In either case, suitable temperature controls must be provided, together with means for optically addressing the individual sample cells 22.

In order to perform an integrated DNA amplification and homogeneous DNA fluorescence polarization assay, the DNA card 20 is placed on the heated carrier of the instrument. The carrier is a heated tray which can be extended outside the instrument to receive the card, and which then withdraws into the instrument in order to perform the desired readings of fluorescence polarization, fluorescence intensity or light absorbance. Typically, the instrument is provided with means for moving the card in both the x and y directions so that each sample cell 22 can be read individually. During the entire operation, the heated carrier maintains the DNA card 20 at an optimum temperature, typically between 25° C and 75° C.

Initially, an empty DNA card 20 is placed on the extended, heated carrier of the instrument and is allowed to equilibrate to the carrier temperature. This equilibration may take approximately one minute. Once the DNA card 20 is equilibrated to the carrier temperature, liquid biological samples 38 are pipetted into the sample ports 26 of one or more of the sample cells 22. The liquid biological samples 38 instantly fill the sample chambers 24 due to a combination

of hydrostatic and capillary force. In the preferred embodiment, the pipetted volume of each liquid biological sample is approximately 20 μL.

As soon as liquid biological samples 38 have been pipetted into all of the sample chambers 24, the sample cells 22 may be sealed using the seals 36, and the measuring instrument may be started. The carrier is then drawn into the instrument for fluorescence polarization, fluorescence intensity or light absorbance reading. Due to the extreme thinness of the sample chambers 24, and the large surface area of the sample chambers 24 with which the liquid biological samples 38 come into contact, the liquid biological samples 38 heat up within seconds of being pipetted into the sample cells 22 to the optimum temperature desired for DNA amplification. Thus, by the time the dried reagent spots 30 dissolve and diffuse throughout the liquid biological samples 38 to begin "priming" of the DNA amplification, the reagents are already up to the optimum temperature. It is in this way that the DNA card 20 effects a "hot start" of the DNA amplification reaction.

Figs. 5 - 7 depict the individual layers of plastic film that the DNA card 20 is composed of. In the preferred embodiment, each layer is approximately 0.015 inch thick. The top layer 44, shown in Fig. 5, contains the holes that form the sample ports 26 and air vents 28. The lower surface of the top layer 44 forms the upper walls of the sample chambers 24. The middle layer 42, shown in Fig. 6, contains keyhole-shaped apertures 54 that form the side walls of the sample chambers 24. The middle layer 42 is coated on both sides with a pressure-sensitive adhesive (not shown). The bottom layer 40, shown in Fig. 7, is a solid rectangular sheet that forms the bottom of the DNA card 20. The upper surface of the bottom layer 40 forms the lower wall of each of the sample chambers 24.

The exploded view of Fig. 8 depicts the relative alignment of the top layer 44, middle layer 42 and bottom layer 40 during assembly of the DNA card 20. In practice, the top layer 44 and middle layer 42 are laminated together with a suitable adhesive, and the partial assembly is then inverted so that the

amplification and assay reagents can be pipetted and dried onto the underside of the top layer 44 to create the dried reagent spots 30. With reference to Fig. 4, the sectioned edge of the DNA card 20 can be seen to include a dried reagent spot 30 that is adhered to the underside of the top layer 44 of the card 20. After the dried reagent spot 30 is formed, the bottom layer 40 is laminated to the middle layer 42, as shown in Fig. 8, to complete the assembly 20.

The dried reagent spot 30 contains both DNA amplification and homogeneous DNA assay reagents, the latter preferably consisting of fluorescence polarization assay reagents. Examples of suitable DNA amplification and DNA fluorescence polarization assay reagents are disclosed in copending U.S. Patent Application Serial No. 08/311,474, filed by G. Terrance Walker et al on September 23, 1995 and entitled "Fluorescence Polarization Detection of Nucleic Acid Amplification", said application being expressly incorporated herein by reference. The chemical reagents in the dried spot 30 are carried in a readily soluble matrix, such a trehalose or another carbohydrate. These reagents will spontaneously re-suspend when exposed to an aqueous sample introduced into the sample chamber 24. It will be understood that more than one dried reagent spot 30 may be provided in each sample cell 22 if desired, as for example by providing the amplification reagents in one spot and the assay reagents in a different spot. In the case of a DNA amplification and homogeneous DNA assay, however, the reagent spots (if separated) should be positioned in such a way that they are dissolved by the liquid biological sample 38 at essentially the same time.

If the homogeneous DNA assay that is to be used in the DNA card 20 is a fluorescence polarization assay, the top layer 44 of the card 20 must be made of a material that does not interfere with the transmission of polarized light. Two examples of materials that satisfy this requirement are cellulose acetate butyrate (CAB) and triacetate cellulose (TAC).

Two alternative embodiments of the sealing strip 32 are illustrated in Figs. 9A and 9B, respectively. In Fig. 9A, the sealing strip 32 is made either of a

transparent CAB having a thickness of about 0.015 inch, with an optically clear pressure-sensitive adhesive (such as Adhesives Research type 8154) applied to its back surface, or of a transparent light-polarizing film with an optically clear pressure-sensitive adhesive applied to its back surface. A suitable adhesive-backed polarizing film is available from Nitto Denko as product number 1220 DU. As previously mentioned, score lines 34 are provided to allow the sealing strip 32 to be separated into individual seals or segments 36 and shown in Fig. 3. In Fig 9B, a modified sealing strip 32' is shown in which each of the segments or seals 36 has a central hole or aperture 56. The holes 56 align with the central circular areas of the sample chambers 24 when the seals 36 are applied to the sample cells 22 as illustrated in Figs. 3 and 4. The sealing strip 32' of Fig. 9B is similar to the sealing strip 32 of Fig. 9A in that it carries a layer of pressure-sensitive adhesive on its back surface, but the sealing strip 32' of Fig. 9B may be made of an opaque material (such as black PVC or CAB) since the holes 56 allow for light transmission to and from the sample chambers 24 through the top layer 44 of the DNA card 20. The sealing strip 32' of Fig. 9B is advantageous in that the light emitted by the liquid biological samples 38 during the fluorescence polarization assay is required to travel only through the top layer 44 of the DNA card 20, rather than through the top layer 44 and the sealing strip 32 as in the embodiment of Fig. 9A.

In fluorescence polarization assays, a polarized excitation beam of a given wavelength of light is used to excite the fluorescent DNA probes. The intensity, at a given wavelength, of fluorescent emission from these excited probes is measured in the plane polarized parallel to the excitation polarization, and also in the plane polarized perpendicular to the excitation polarization. When a fluorescent DNA probe hybridizes to a DNA amplicon, the intensity of fluorescent emission in the plane parallel to the excitation plane increases. Typically, both parallel and perpendicular intensities are measured. The changes in total intensity are then compensated for by applying the formula:

$$P = (I_{PARA} - I_{PER}) / (I_{PARA} + I_{PER}),$$

where:

I_{PARA} = Fluorescent intensity in the plane polarized in the plane polarized parallel to the plane of excitation polarization; and

I_{PER} = Fluorescent intensity in the plane polarized in the plane polarized perpendicular to the excitation polarization.

This formula yields the dimensionless quantity referred to as the polarization ratio (P).

Since it is the polarization intensity in the plane parallel to the excitation polarization which increases with increased hybridization, measuring the intensity of the polarization in the plane parallel to the excitation polarization over time will show the increase in hybridization over time. This is a kinetic or dynamic approach to the measurement of fluorescence polarization, which is also suitable for use with fluorescence energy transfer and light absorbance assays. By using such a kinetic or dynamic approach, compensation for absolute intensity becomes somewhat less important because each sample is measured against itself and is thus a relative measurement. In the case of a fluorescence polarization assay, therefore, it becomes necessary to measure fluorescence intensity only in the plane polarized parallel to the plane of the excitation polarization.

The kinetic or dynamic approach described above allows for the use of a confocal polarization method, where the polarizer for the excitation beam is also used as the polarizer for the fluorescence emitted by the sample, thereby reducing the number of required polarizing elements to one. This differs from the conventional approach, in which separate polarization elements are needed in the measuring instrument for both the excitation beam and the sensor used to detect the fluorescent emissions from the samples. With only a single polarizing element being required in the confocal method, this element can be provided in

the DNA card 20 itself (i.e., in the form of a polarized sealing strip 32 or top layer 44) and need not be provided in the measuring instrument as in the prior art. Thus, standard microplate fluorometers containing no polarization elements can be used in the fluorescence polarization assay of the present invention. In the case of fluorescence energy transfer assays, standard microplate fluorometers can also be used, and in the case of light absorption assays, standard microplate readers can be used.

The enlarged cross-sectional view of Fig. 10 illustrates the sample chamber 24 of one sample cell 22 filled with a liquid biological sample 38 during a fluorescence polarization assay. In this example, the seal 36 is made of a plastic polarizing film, and serves as the confocal polarizer during the assay. The top layer 44 of the DNA card 20 is made of transparent, non-polarizing CAB. In operation, an unpolarized light beam 58 is directed toward the sample cell 22 containing the liquid biological sample 38. When the unpolarized light beam passes through the polarizing seal 36, the transmitted light beam 60 is of single polarization. The fluorescent DNA probes in the liquid biological sample 38 are excited by the polarized beam 60 and emit light (indicated by the arrow 62) of various polarizations. However, the same polarizing seal 36 polarizes these emissions in a plane parallel to that of the excitation beam 60, resulting in a polarized beam 64 being detected by the fluorometer. In this way, a confocal polarization method is implemented without requiring any polarization elements in the fluorometer itself.

Fig. 11 is a graph of the typical relationship that homogeneous DNA amplification and assay reactions exhibit with respect to time. This relationship is similar whether assay reagents for fluorescence polarization, fluorescence energy transfer (fluorescence intensity) or light absorbance are used. The graph shows that the reactions exhibit an initial exponential portion 66, and a final linear portion 68. Fig. 12 depicts a graph similar to that of Fig. 11, but includes curves for three different concentrations of genomes. The concentrations used are

10 genomes, 100 genomes and 1000 genomes per unit volume. Typical DNA probe assays are performed on fully amplified samples, in which case they are end point reactions. DNA amplification reactions typically produce a maximum number of amplicons that is independent of the starting number of genomes. In Fig. 12, it can be seen that the 1000-genome curve exhibits its exponential phase 70 at a time before the 100-genome curve exhibits its exponential phase 72. Similarly, the 100-genome curve exhibits its exponential phase 72 at a time before the 10-genome curve exhibits its exponential phase 74. However, by the time labelled $t_{end\ point}$, the magnitudes of all three curves are very similar and there is only a small difference between the 1000-genome, 100-genome and 10-genome curves, as shown by the points *a*, *b* and *c* on the vertical axis. By accumulating fluorescence intensity data during the entire time interval represented by any given one of the curves in Fig. 12 (i.e., between t_0 and $t_{end\ point}$), rather than simply taking the final reading at $t_{end\ point}$, information about the fluorescence polarization assay is available earlier and with better resolution. In addition, various different protocols may be used. For instance, by measuring the time to a given amplitude (point *d* in Fig. 12), it can be seen that the 1000-genome curve at time t_1 will be detected first and that its resolution from the 100-genome curve (at time t_2) is increased, as is the resolution of the 100-genome curve from that of the 10-genome curve at time t_3. Alternatively, examining the amplitudes of the three curves over time indicates that there are many places better than $t_{end\ point}$ to make measurements to resolve the differences in the three curves. If time t_1 is taken, for example, there is much better resolution between the 1000-genome curve and the 100-genome curve than at $t_{end\ point}$, and a similar increase in resolution exists between the 100-genome curve and the 10-genome curve at time t_2.

As noted previously, the nature of the measuring instrument with which the DNA card 20 is used will vary depending upon the construction of the DNA card 20 itself. For embodiments of the DNA card 20 containing polarizing

elements, a typical microplate fluorometer with suitable thermal control can be used. For embodiments of the DNA card 20 that do not contain polarizing elements, a measuring instrument containing such elements in required. An example of such an instrument is shown in Figs. 13 - 15.

Referring first to Fig. 13, the measuring instrument 80 is shown in a perspective view with a portion cut away to illustrate the manner in which the DNA card 20 is received in the instrument. The DNA card 20 is initially placed on a heated carrier 81 which extends out over the front portion 82 of the instrument, and is then drawn automatically into the interior of the instrument at the position shown. In this position, shown in more detail in the enlarged view of Fig. 14, the DNA card 20 is located below three polarized laser diode sources 84 of different wavelengths such as 630 nm, 660 nm and 690 nm. A motor 86 selectively rotates a six-position polarized filter wheel 88 to position polarized wavelength filters 90, 92, 94 and 96 (and two additional filters which are not visible) above the sample cell 22 of interest. These filters match the emissions of the various fluorescent DNA probes and allow for the detection and measurement of these wavelengths by a photomultiplier tube (PMT) detector 98 in planes both parallel and perpendicular to the polarization plane of the input or source beam. The heated carrier 81 is indexed in the x and y directions (by means not shown) to address different ones of the sample cells 22.

Fig. 15 is a front or edge-on view of the DNA card 20 of Figs. 13 and 14, showing a single source 84 which provides a specular input beam 100 directed to a particular sample cell 22 of the DNA card 20. This beam is monochromatic and polarized as a consequence of having been generated by a laser diode source. If other types of sources are used, a polarizer and wavelength filter are required. When excited by the input beam 100, the fluorescent DNA probes in the sample cell 22 of the DNA card 20 emit light 102. The light 102 passes through the polarizer and wavelength filter of the filter wheel 88, to the PMT photodetector 98 for detection and measurement.

Examples of several different ways in which the DNA card 20 may be constructed are provided below. It should be understood that these examples are merely illustrative and are not intended to limit the scope of the present invention.

Example 1

The DNA card 20 comprises a top layer 44 made of transparent, non-polarizing CAB, a middle layer 42 made of black PVC, and a bottom layer 40 made of transparent, non-polarizing CAB. The sealing strips 32 have the configuration shown in Fig. 9A, and are made of a plastic polarizing film coated on one side with an optically clear pressure-sensitive adhesive. The resulting DNA card 20 can be used in a standard microplate fluorometer having no polarizing elements.

Example 2

The DNA card 20 comprises a top layer 44 is made of a plastic polarizing film, a middle layer 42 made of black PVC, and a bottom layer 40 made of transparent, non-polarizing CAB. The sealing strips 32' are made of black PVC and have the configuration illustrated in Fig. 9B. The resulting DNA card 20 can be used in a standard microplate fluorometer having no polarizing elements.

Example 3

The top layer 44, middle layer 42 and bottom layer 40 of the DNA card 20 are all made of transparent, non-polarizing CAB. The sealing strips 32 have the configuration shown in Fig. 9A, and are made of a transparent, non-polarizing CAB with an applied pressure-sensitive adhesive. When constructed with fluorescence polarization assay reagents, the resulting DNA card 20 is used in a measuring instrument containing polarizing elements as illustrated, for example, in Figs. 13 - 15. Alternatively, this embodiment, when constructed with

fluorescence energy transfer assay reagents, results in a DNA card 20 that is measured on a typical microplate fluorometer. This embodiment is also suitable for construction with light absorbance assay reagents, which can be measured on a typical microplate reader.

Example 4

The DNA card 20 comprises a top layer 44 made of transparent, non-polarizing CAB, and a middle layer 42 and bottom layer 40 both made of black CAB. The sealing strips 32 have the configuration shown in Fig. 9A, and are made of a plastic polarizing film with an applied pressure-sensitive adhesive. The resulting DNA card 20 may be used with a conventional microplate fluorometer.

Example 5

The top, middle and bottom layers 44, 42 and 40 of the DNA card 20 are as set forth in Example 4. However, the sealing strips 32' have the configuration shown in Fig. 9B, and are made of transparent, non-polarizing CAB with an applied pressure-sensitive adhesive. The resulting DNA card 20 is used in a measuring instrument of the type illustrated in Figs. 13 - 15.

Example 6

The top layer 44, middle layer 42 and bottom layer 40 of the DNA card 20 are as set forth in Example 3, and the sealing strips 32 have the configuration shown in Fig. 9a. However, the sealing strips 32 are made of a plastic polarizing film with an applied pressure-sensitive adhesive. The resulting DNA card 20 may be used with a conventional microplate fluorometer.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof, as numerous alternatives to the devices and methods described which incorporate the present invention will be apparent to

those skilled in the art. The invention is accordingly defined by the following claims with equivalence of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. An apparatus for carrying out a homogeneous nucleic acid amplification and nucleic acid assay on a liquid biological sample, comprising:

a sample cell for receiving a liquid biological sample, said sample cell having a sample chamber and a sample port for admitting said liquid biological sample into said sample chamber; and

a dried nucleic acid amplification reagent and a dried homogeneous nucleic acid assay reagent, said reagents being adhered to the interior of said sample chamber for reacting with said liquid biological sample.

2. An apparatus as claimed in claim 1, wherein said dried nucleic acid amplification reagent and said homogeneous nucleic acid assay reagent are provided in the form of a single spot adhered to an internal surface of said sample chamber.

3. An apparatus as claimed in claim 1, further comprisinga sealing member attachable to said sample cell for sealing said sample port after said liquid biological sample has been admitted to said sample chamber.

4. An apparatus as claimed in claim 1, wherein said homogeneous nucleic acid assay reagent comprises a fluorescence polarization assay reagent, and wherein at least a portion of said sample cell is sufficiently light-transmissive to permit external detection of a fluorescence polarization reaction occurring in a liquid biological sample contained in said sample chamber.

5. An apparatus as claimed in claim 1, wherein:

said homogeneous nucleic acid assay reagent comprises a fluorescence polarization assay reagent;

at least a portion of said sample cell is sufficiently light-transmissive to permit external detection of a fluorescence polarization reaction occurring in a liquid biological sample contained in said sample chamber; and

said sealing member is made of a light-transmissive material and is attachable over said light-transmissive portion of said sample chamber.

6. An apparatus as claimed in claim 5, wherein at least one of said sealing member and said light-transmissive portion of said sample chamber is made of a light-polarizing material.

7. An apparatus as claimed in claim 1, wherein said sample cell further comprises an air vent for allowing air to be displaced from said sample chamber during admission of said liquid biological sample into said sample cell.

8. An apparatus as claimed in 1, wherein:

said sample cell is formed in a substantially flat, laminated card-like member having a bottom layer forming a bottom wall of said sample chamber, an apertured middle layer forming side walls of said sample chamber, and a top layer forming an upper wall of said sample chamber, said sample port being formed in said top layer; and

said sample cell is one of a plurality of substantially identical sample cells disposed in a two-dimensional array across the length and width of said card-like member.

9. An apparatus as claimed in claim 8, further comprising a sealing member for sealing the sample ports of said sample cells after liquid biological samples have been admitted to said sample chamber, said sealing member comprising a layer of flexible material which is attachable to the top layer of said laminate by

means of a pressure-sensitive adhesive carried on the underside of said layer of flexible material.

10. An apparatus for carrying out a biological process on a liquid biological sample, comprising:

a substantially flat, card-like member having at least one sample cell therein for receiving a liquid biological sample, said sample cell having a sample chamber, sample port for admitting said liquid biological sample into said sample chamber, an air vent for allowing air to be displaced from said sample chamber during admission of said liquid biological sample into said sample chamber and a dried reagent adhered to an internal surface of said sample chamber for reacting with said liquid biological sample; and

a sealing member attachable to said card-like member for sealing said sample port and said air vent after said liquid biological sample has been admitted to said sample chamber.

11. An apparatus as claimed in claim 10, wherein said card-like member has opposed top and bottom surfaces, and wherein said sample port and said air vent are both formed in the top surface of said card-like member.

12. An apparatus as claimed in claim 10, wherein said card-like member comprises a laminate having a bottom layer forming a bottom well of said sample chamber, an apertured middle layer forming side walls of said sample chamber, and a top layer forming an upper wall of said sample chamber, said sample port and said air vent being formed in said top layer.

13. An apparatus as claimed in claim 12, wherein said sealing member comprises a layer of flexible material which is attachable to the top layer of said

laminate by means of a pressure-sensitive adhesive carried on the underside of said layer of flexible material.

14. An apparatus as claimed in claim 13, wherein said dried reagent reacts with said liquid biological sample to produce an optical indication, and wherein said sealing member and said top layer are sufficiently light-transmissive in the region above said sample chamber to permit external detection of said optical indication.

15. An apparatus as claimed in 14, wherein said dried reagent comprises a nucleic acid fluorescence polarization assay reagent.

16. An apparatus as claimed in claim 10, wherein said sample cell has a generally elongated shape in a direction parallel to the plane of said card-like member, with said sample port located at one end of said sample cell and said air vent located at the opposite end thereof.

17. An apparatus as claimed in claim 10, wherein said sample cell is one of a plurality of substantially identical sample cells disposed in a two-dimensional array across the length and width of said card-like member.

18. An apparatus for carrying out a nucleic acid fluorescence polarization assay on a liquid biological sample, comprising:

a sample cell for receiving a liquid biological sample, said sample cell having a sample chamber and a sample port for admitting said liquid biological sample into said sample chamber; and

a dried nucleic acid fluorescence polarization assay reagent adhered to an internal surface of said sample chamber for reacting with said liquid biological sample;

wherein at least a portion of said sample cell is made of a light-transmissive, light-polarizing material to facilitate external detection of a fluorescence polarization reaction occurring in a liquid biological sample contained in said sample chamber.

19. An apparatus as claimed in claim 18, wherein said portion of said sample cell comprises a sealing member attachable to said sample cell for sealing said sample port after said liquid biological sample has been admitted to said sample chamber.

20. An apparatus as claimed in claim 18, wherein:

said sample cell is formed in a substantially flat, laminated card-like member having a bottom layer forming a bottom wall of said sample chamber, an apertured middle layer forming side walls of said sample chamber, and a top layer forming an upper wall of said sample chamber, said sample port being formed in said top layer; and

said portion of said sample cell comprises the top layer of said laminated card-like member.

21. An apparatus as claimed in claim 20, wherein said sample cell is one of a plurality of substantially identical sample cells disposed in a two-dimensional array across the length and width of said card-like member.

22. An apparatus as claimed in claim 18, further comprising a dried nucleic acid amplification reagent adhered to an internal surface of said sample chamber for reacting with said liquid biological sample.

23. A method for carrying out an integrated nucleic acid amplification and homogeneous nucleic acid fluorescence polarization assay on a liquid biological sample, comprising the steps of:

introducing a liquid biological sample into a sample cell having a light-transmissive portion;

bringing said liquid biological sample into contact with a dried nucleic acid amplification reagent and a dried homogeneous nucleic acid fluorescence polarization assay reagent within said sample cell;

sealing said sample cell;

incubating said sample cell to allow said liquid biological sample to react with said nucleic acid amplification reagent and said homogeneous nucleic acid fluorescence polarization assay reagent; and

detecting fluorescence polarization in said liquid biological sample through said light-transmissive portion of said sample cell.

24. A method as claimed in claim 23, wherein the step of detecting fluorescence polarization in said liquid biological sample comprises directing polarized light through said light-transmissive portion of said sample cell.

25. A method as claimed in claim 23, wherein the light-transmissive portion of said sample cell is made of a light-polarizing material, and wherein the step of detecting fluorescence polarization in said liquid biological sample comprises directing unpolarized light through said light-transmissive portion.

26. A method for carrying out carrying out a nucleic acid amplification reaction on the liquid biological sample, comprising the steps of:

preheating a sample cell containing a dried nucleic acid amplification reagent to a temperature suitable for nucleic acid amplification;

introducing a liquid biological sample into said preheated sample cell to bring said liquid biological sample into contact with said dried nucleic acid amplification reagent;

equilibrating the temperature of said liquid biological sample to the temperature of said preheated sample cell; and

after said temperature equilibration is substantially complete, commencing said nucleic acid amplification reaction in said sample cell.

27. A method as claimed in claim 26, wherein the step of equilibrating the temperature of said liquid biological sample to the temperature of said preheated sample cell comprises forming a thin layer of said liquid biological sample in said sample cell to enhance heat transfer between said sample cell and said liquid biological sample.

28. A method as claimed in claim 26, wherein the time required for said temperature equilibration to occur is substantially the same as the time required for said liquid biological sample to dissolve said dried nucleic acid amplification reagent.

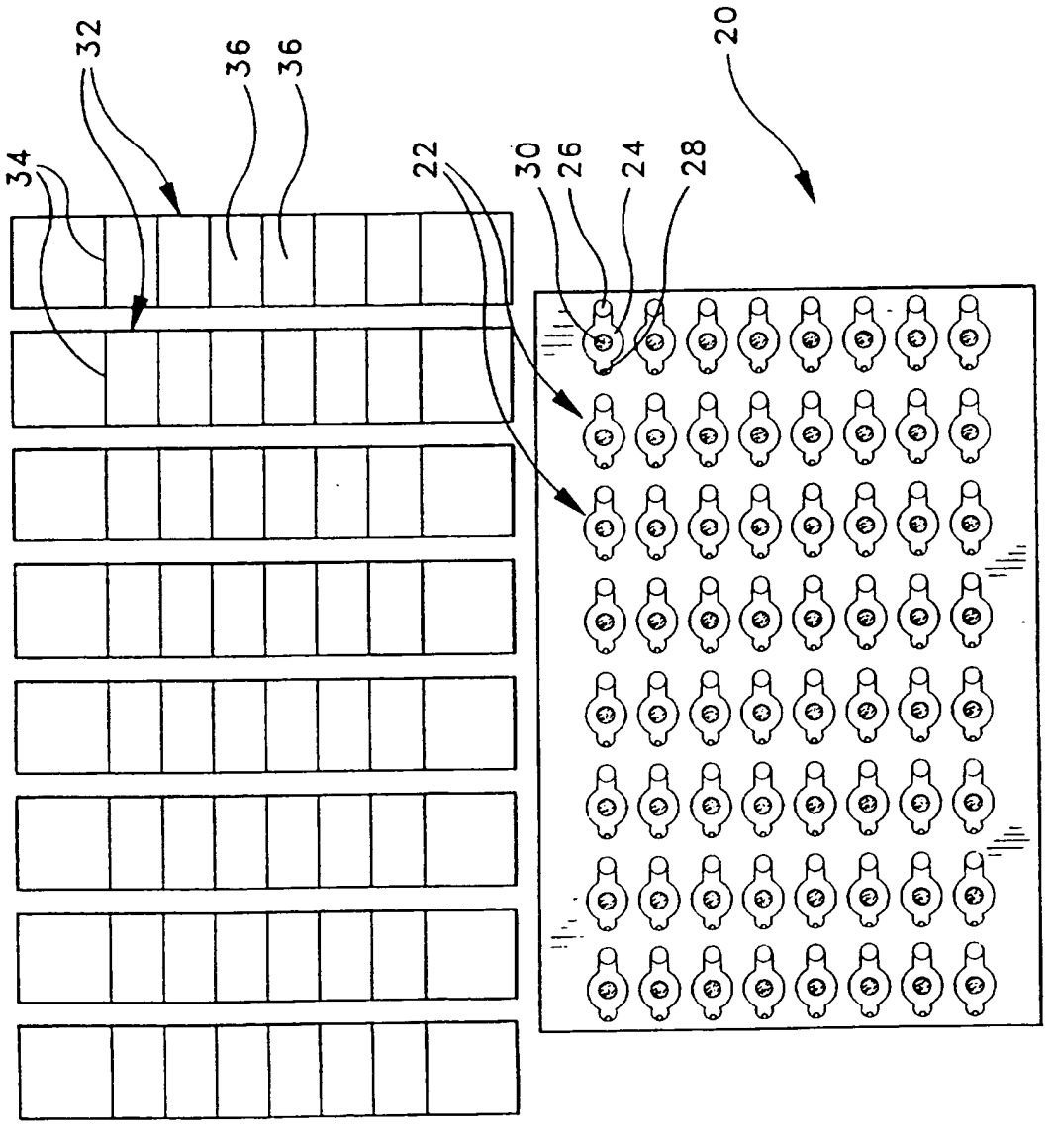


FIG-1

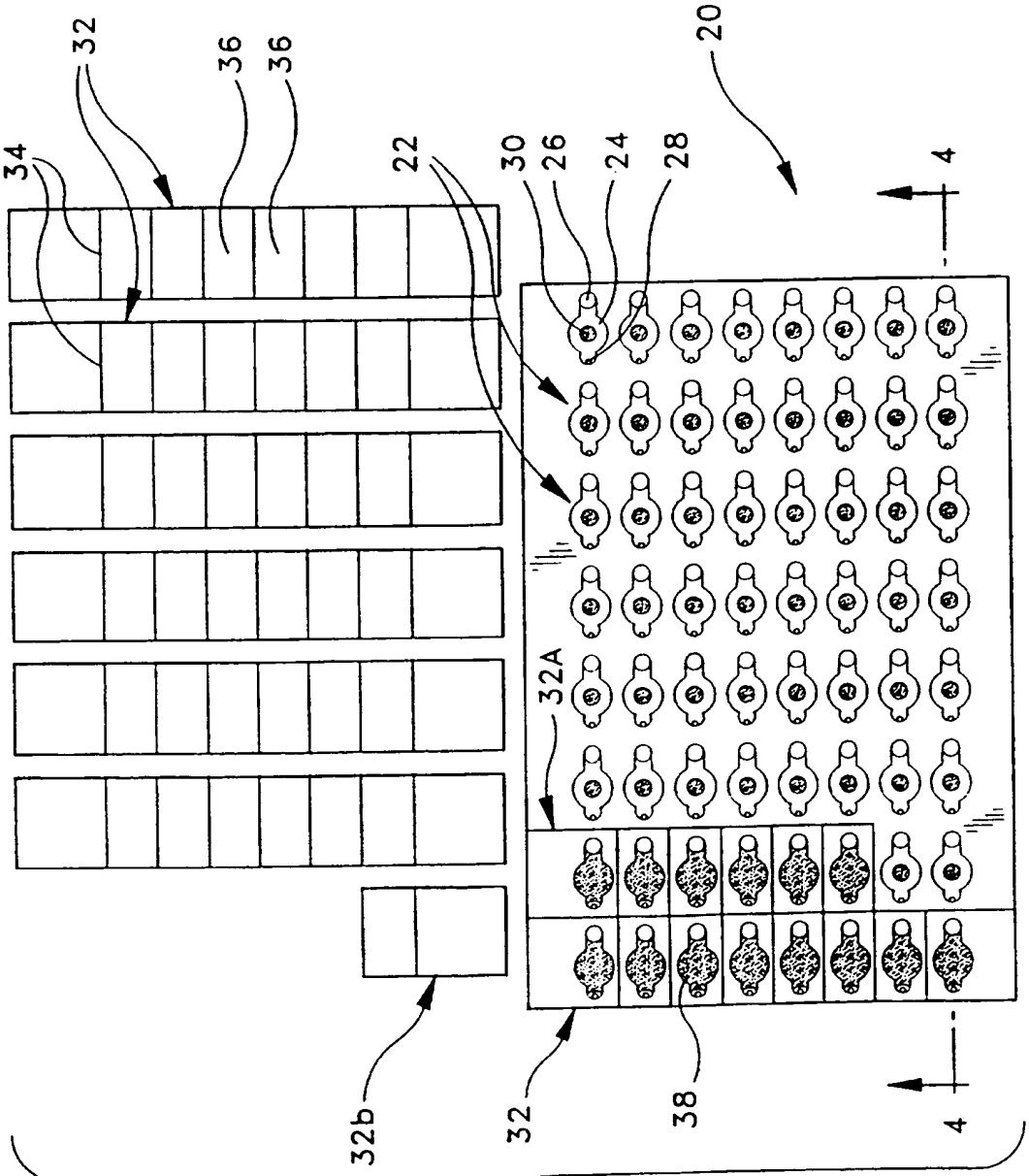


FIG-3

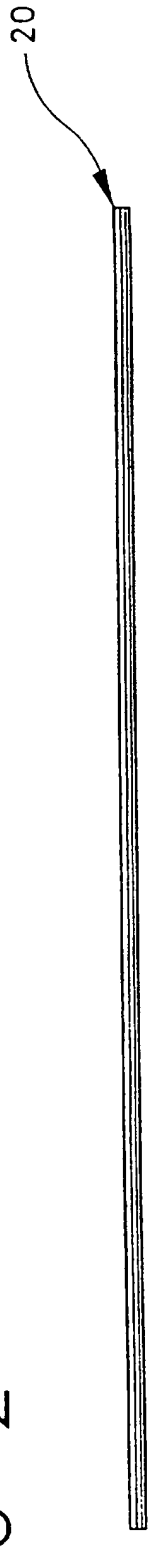


FIG-2

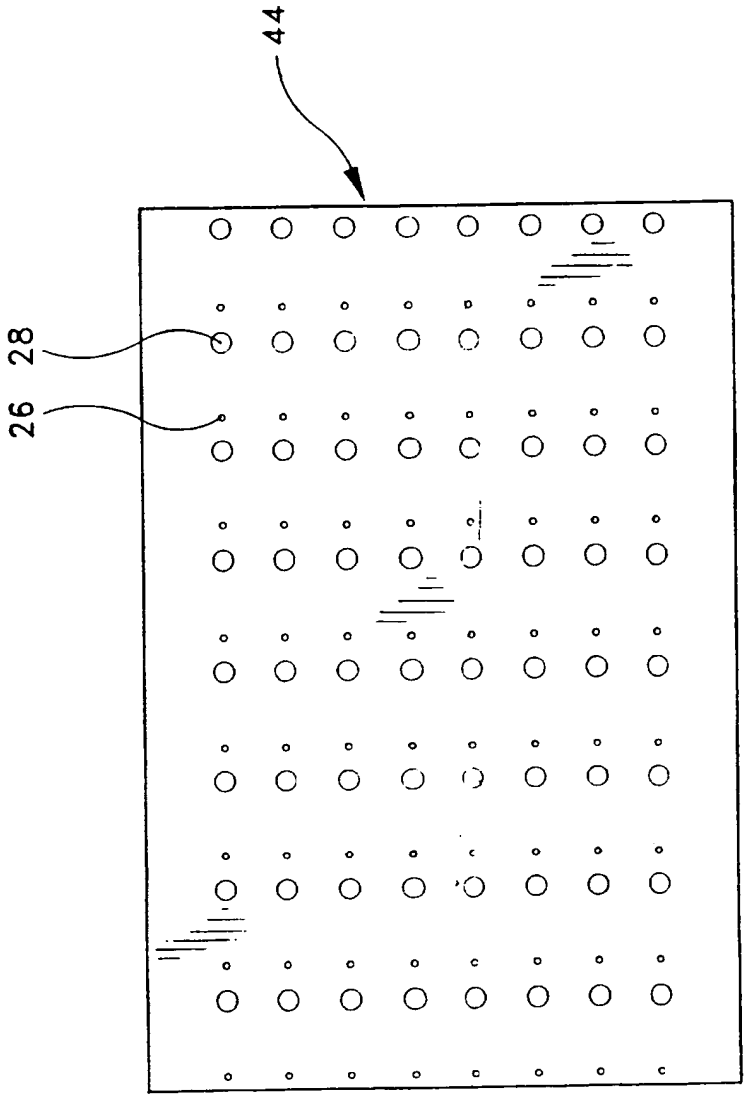


FIG-5

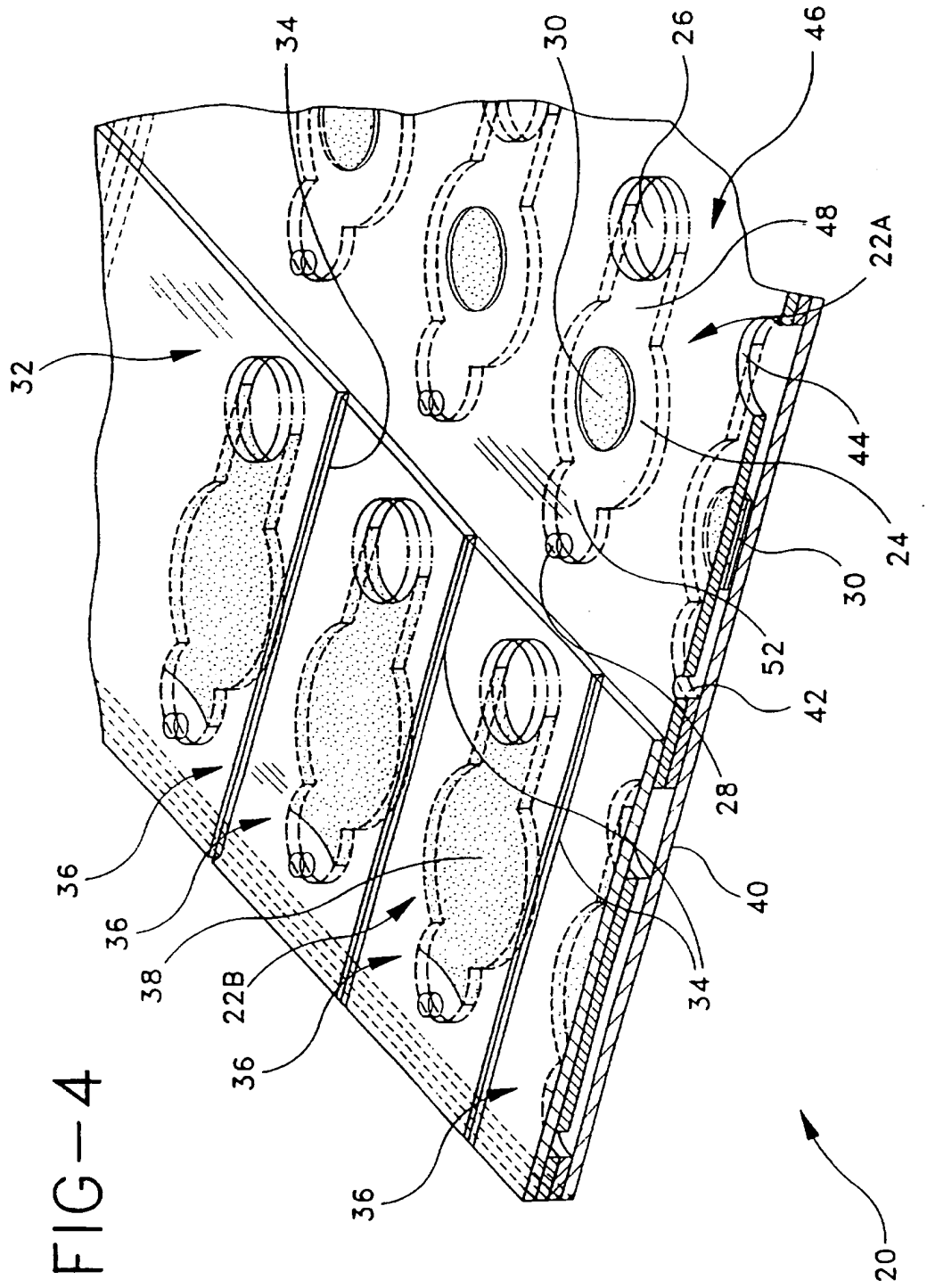


FIG-4

FIG-6

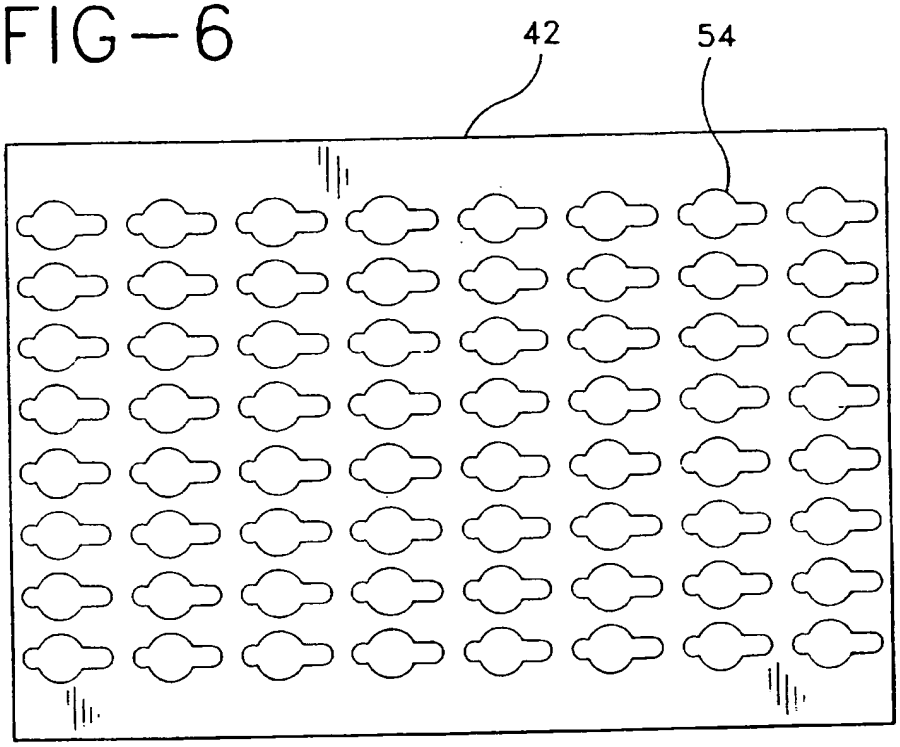


FIG-7

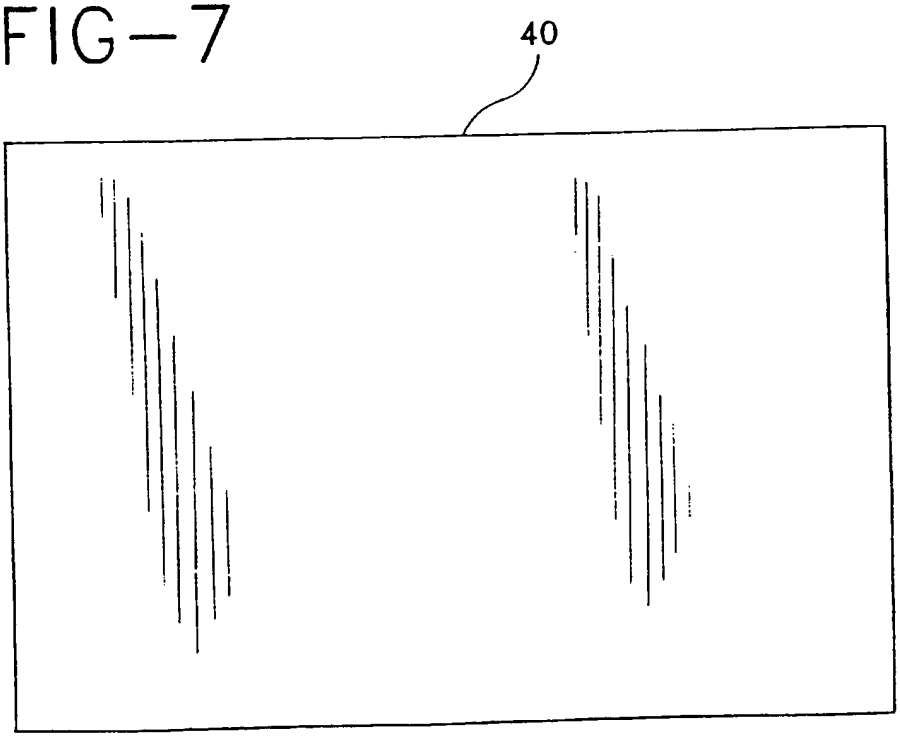
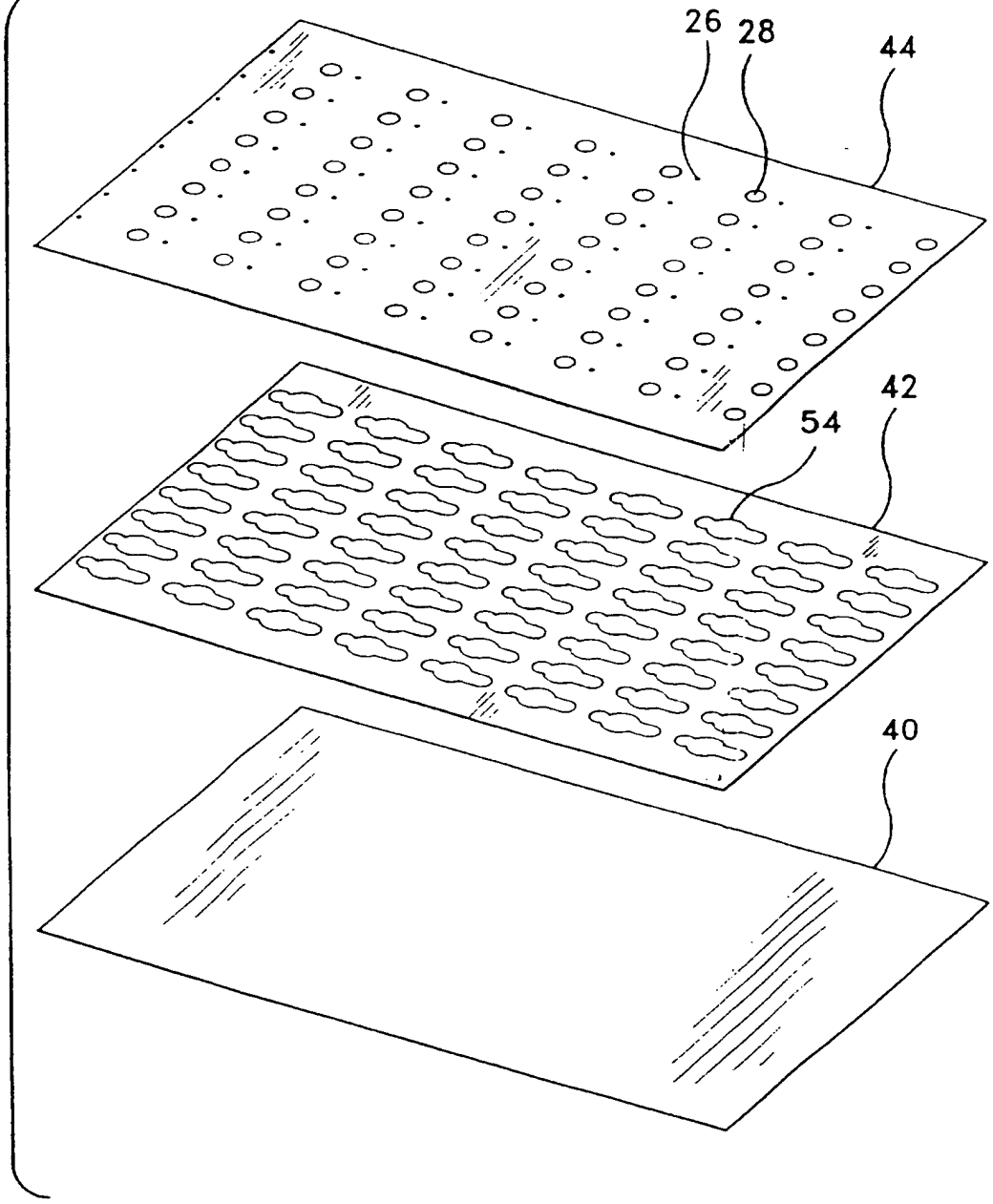


FIG-8



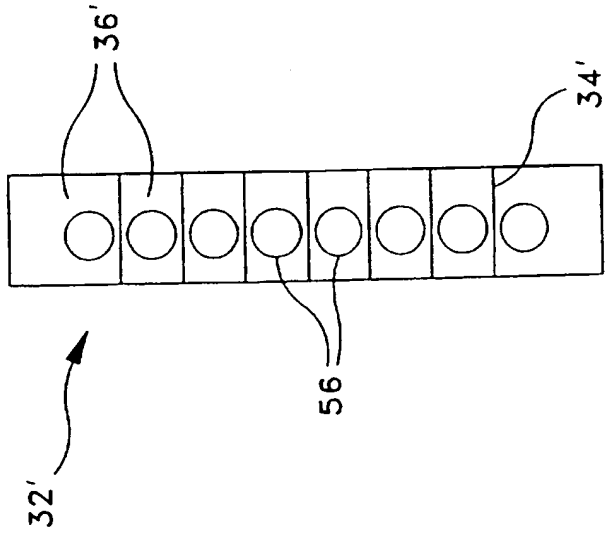


FIG-9B

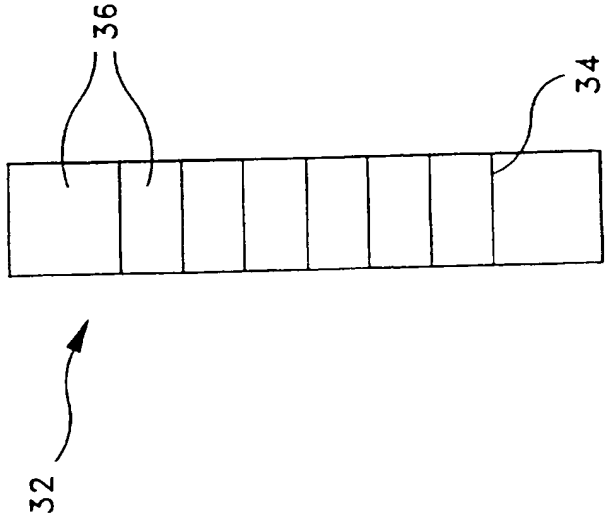


FIG-9A

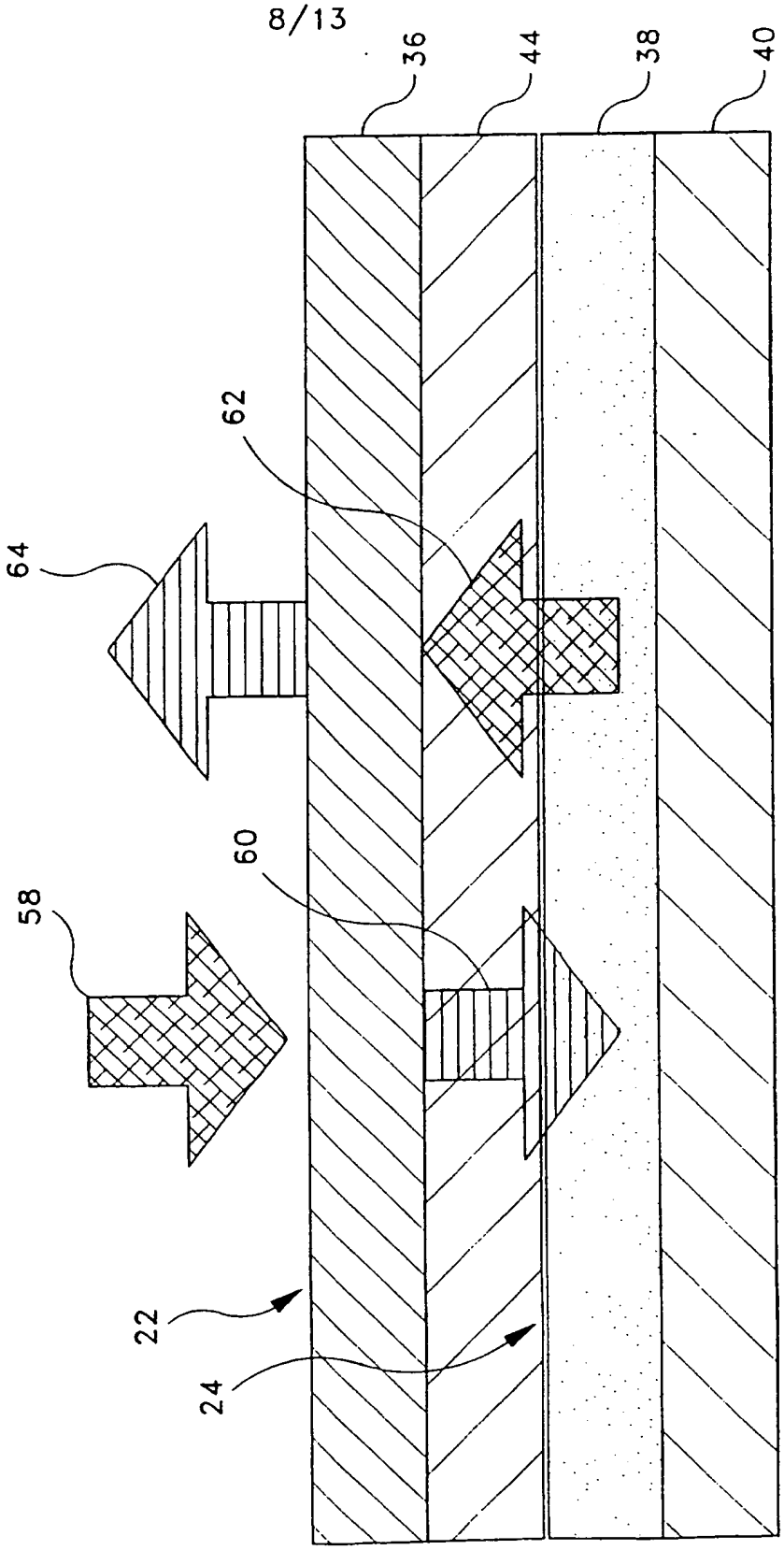


FIG-10

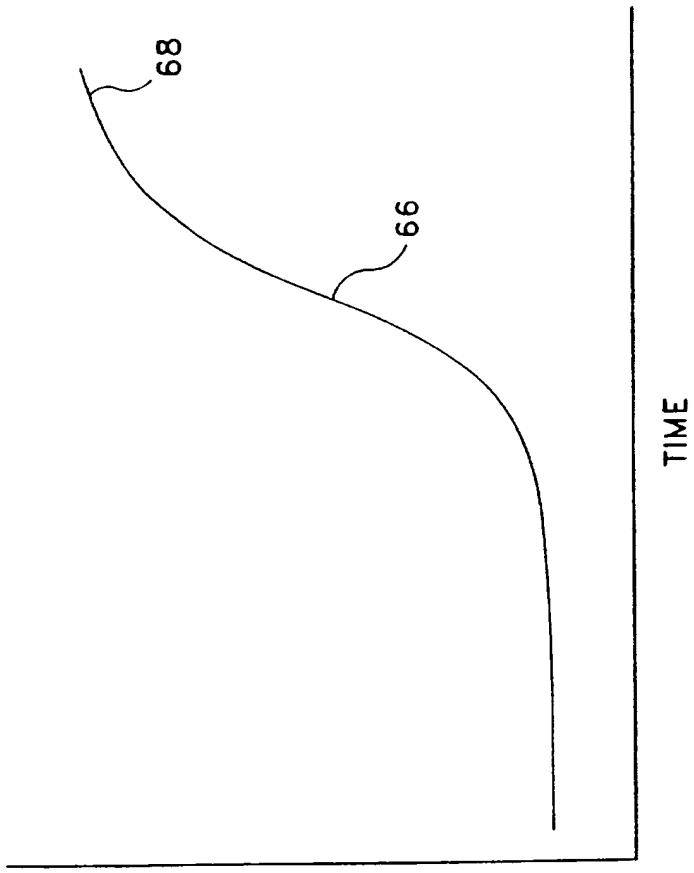

68
66
TIME
DNA AMPLIFICATION
FLUORESCENCE POLARIZATION
OR
FLUORESENCE INTENISTY

FIG-11

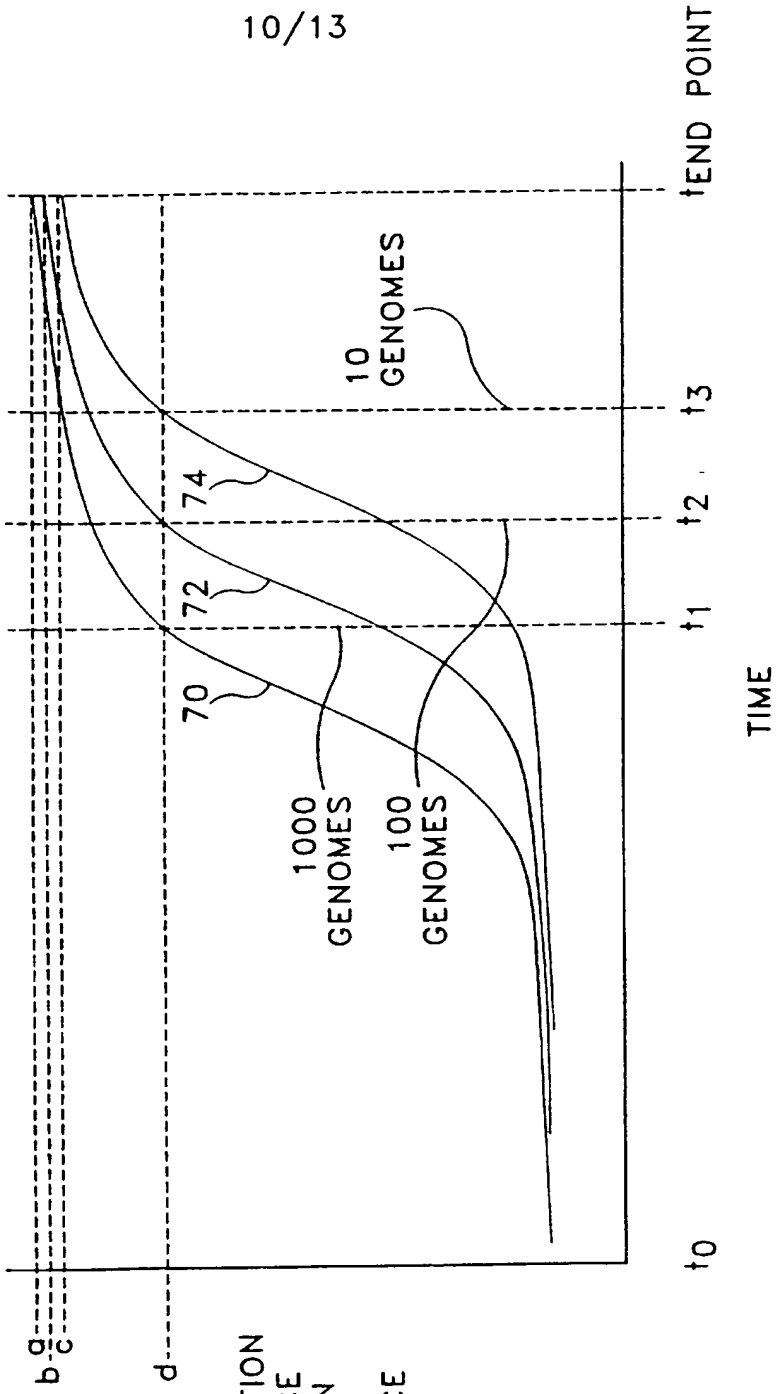

DNA AMPLIFICATION
FLUORESCENCE
POLARIZATION
OR
FLUORESCENCE
INTENSITY
TIME
t0
t1
t2
t3
tEND POINT
a
b
c
d
70
72
74
1000
GENOMES
100
GENOMES
10
GENOMES

FIG-12

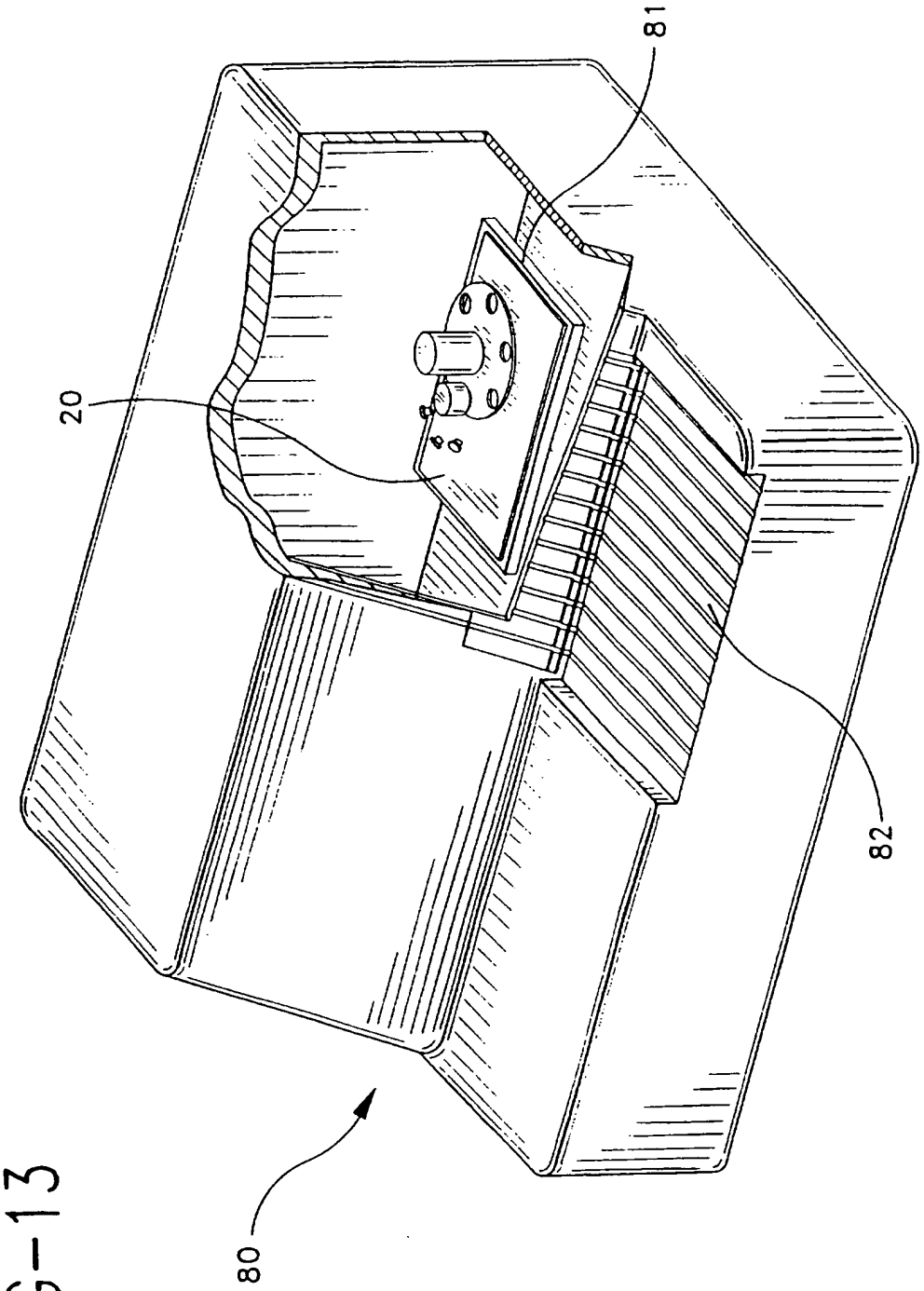


FIG-13

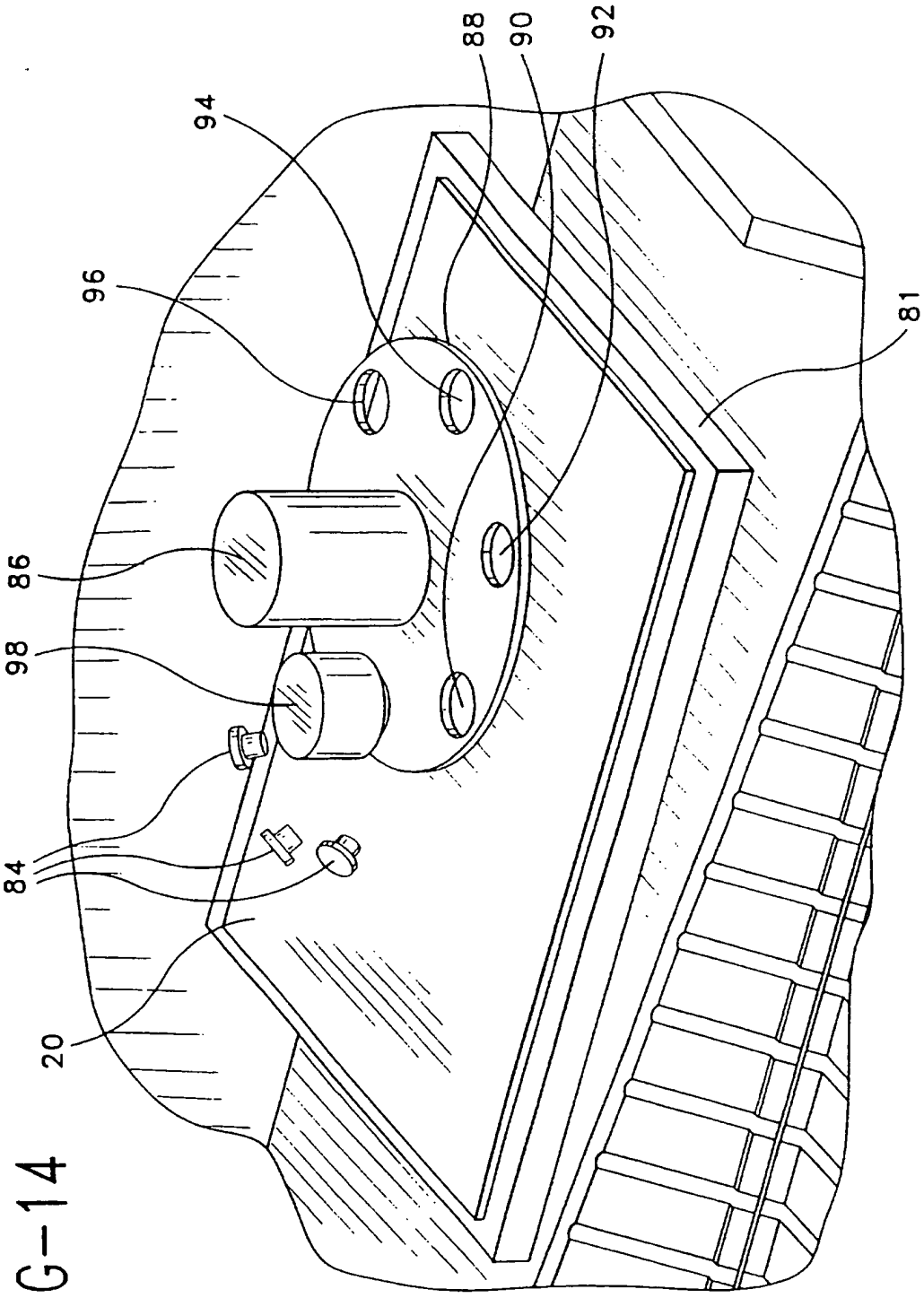


FIG-14

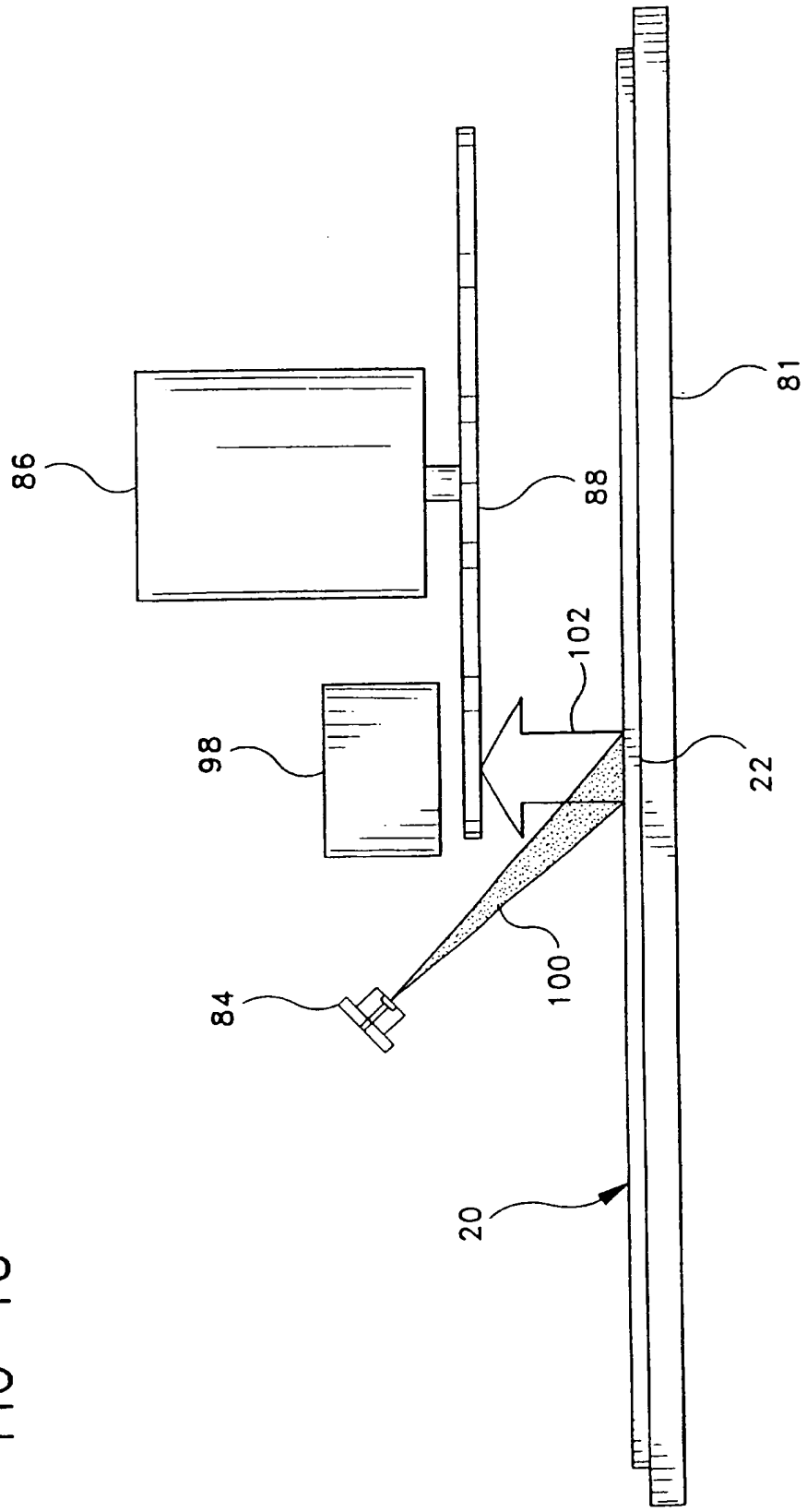


FIG-15

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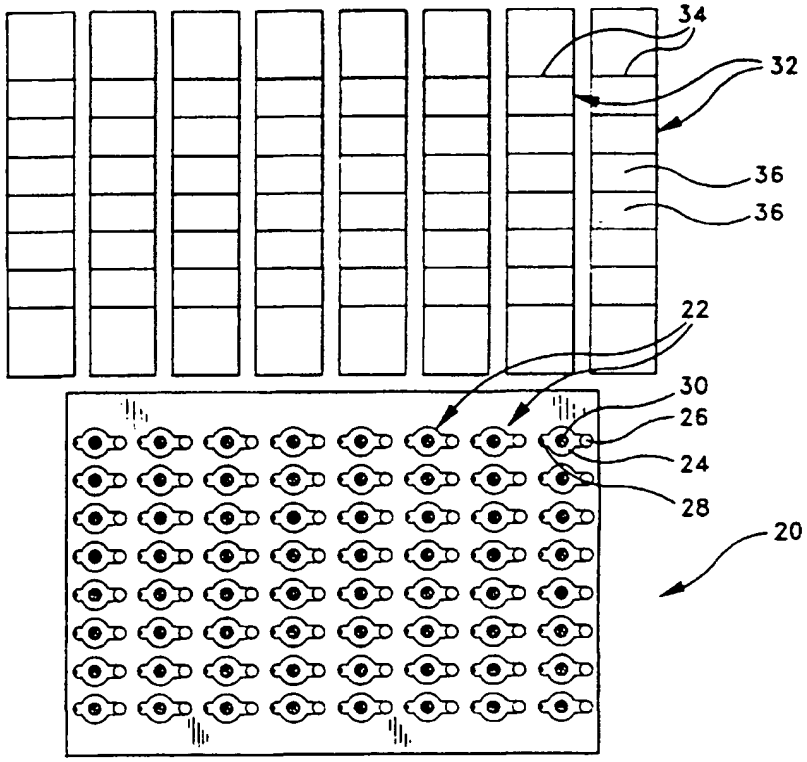
(57) Abstract

A DNA amplification and homogeneous DNA probe assay device is provided which includes a multiplicity of discrete sample cells in a flat "card" format, with each sample cell containing the reagents necessary for both DNA amplification and homogeneous DNA probe assay. The device is particularly suitable for fluorescence polarization DNA probe assays, and is preferably provided with an integral polarizer to avoid the need for polarizing elements in the related measuring apparatus. The size and geometry of the sampe cells allows for a "hot start" of the DNA amplification reaction and thereby avoids mispriming of the amplification reaction.



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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 96/14681

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/00 B01L7/00 C12Q1/68 G01N21/03 G01N21/64
G01N21/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 16194 A (DIAGEN INST FUER MOLEKULAR BIO) 19 August 1993	1-3,9
Y	see page 7, last paragraph - page 8, paragraph 1 see page 13, last paragraph - page 14, paragraph 2 see page 15, paragraph 3 - page 16, paragraph 1; figure 1	4,23,24
Y	see page 23, last paragraph - page 25, paragraph 1 ---	10-14, 16,17
Y	EP 0 382 433 A (ICI PLC) 16 August 1990 see page 3, line 2 - line 36 see page 10, line 22 - page 11, line 37 --- -/--	4,23,24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 March 1997	16.04.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hocquet, A

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INTERNATIONAL SEARCH REPORT

In ational Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 215 419 A (MILES) 25 March 1987 see page 4, line 19 - page 5, line 6 see page 10, line 12 - page 12, line 3; figures	10-14, 16,17
A	EP 0 318 255 A (EASTMAN KODAK CO) 31 May 1989 see column 2, line 16 - line 45 see column 7, line 46 - column 8, line 35; figure 10	10,11,27
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In ational Application No
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INTERNATIONAL SEARCH REPORT

.ternational application No.
PCT/US 96/ 14681

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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2. ☐ Claims Nos.:
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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. CLAIMS 1 - 25
2. CLAIMS 26 - 28

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1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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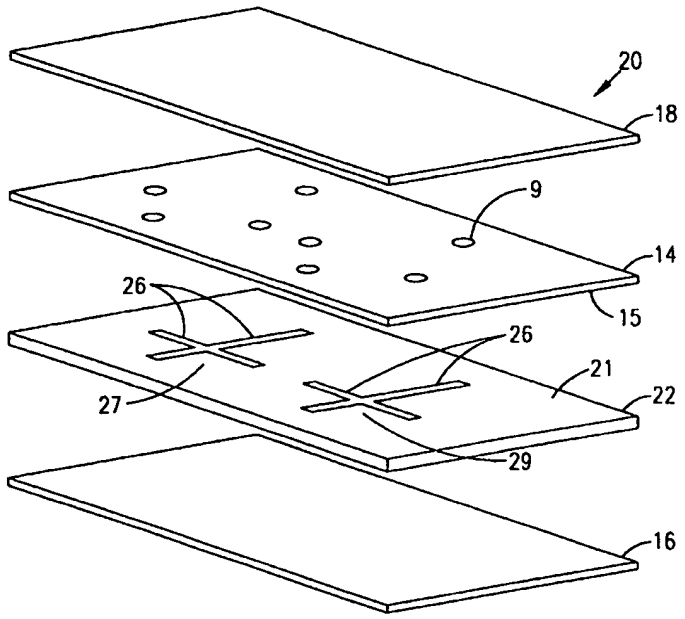
(54) Title: LAMINATE MICROSTRUCTURE DEVICE AND METHOD FOR MAKING SAME



(57) Abstract

A continuous form microstructure array device (20) is constructed as a flexible elongate film laminate containing microstructure arrays (26) arranged serially along the laminate. The laminate can be continuously drawn from a roll, passed through a processing and analysis device and rerolled or stacked for storage.

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LAMINATE MICROSTRUCTURE DEVICE AND METHOD FOR MAKING SAME

BACKGROUND

This invention relates to methods and apparatus for high throughput sample analysis.

In a range of technology-based industries, including the chemical, bioscience, biomedical, and pharmaceutical industries, it has become increasingly desirable to develop capabilities for rapidly and reliably carrying out chemical and biochemical reactions in large numbers using small quantities of samples and reagents. Carrying out a massive screening program manually, for example, can be exceedingly time consuming and may be entirely impracticable where only a very small quantity of an important sample or component of interest is available, or where a component of a synthesis or analysis is very costly.

Developments in a variety of fields have resulted in an enormous increase in the numbers of targets and compounds that can be subjected to screening.

Rapid and widespread advances in the scientific understanding of critical cellular processes, for example, has led to rationally designed approaches in drug discovery. Molecular genetics and recombinant DNA technologies have made possible the isolation of many genes, and the proteins encoded by some of these show promise as targets for new drugs. Once a target is identified and the gene is cloned, the recombinant protein can be produced in a suitable expression system. Often receptors and enzymes exist in alternative forms, subtypes or isoforms, and using a cloned target focuses the primary screen on the subtype appropriate for the disease. Agonists or antagonists can be identified and their selectivity can then be tested against the other known subtypes. The availability of such cloned genes and corresponding expression systems require screening methods that are specific, sensitive, and capable of automated very high throughput.

Similarly, an emergence of methods for highly parallel chemical synthesis has increased the need for high throughput screening ("HTS"). Conventionally, preparation of synthetic analogs to the prototypic lead compound was the established method for drug discovery. Natural products were usually isolated from soil microbes and cultured under a wide variety of conditions. The spectrum of organisms employed by the pharmaceutical industry for isolation of natural products has now expanded from actinomycetes and fungi to include plants, marine organisms, and insects. More recently, the chemistry of creating combinatorial libraries has vastly increased the number of synthetic compounds available for testing. Thousands to tens or hundreds of thousands of small molecules can be rapidly and economically synthesized. *See,*

e.g., U.S. Patent No. 5,252,743 for a discussion of combinatorial chemistry. Thus, combinatorial libraries complement the large numbers of synthetic compounds available from the more traditional drug discovery programs based, in part, on identifying lead compounds through natural product screening.

Accordingly, considerable resources have been directed to developing methods for high-throughput chemical syntheses, screening, and analyses. A considerable art has emerged, in part from such efforts.

Competitive binding assays, originally developed for immunodiagnostic applications, continue to be commonly employed for quantitatively characterizing receptor-ligand interactions. Despite advances in the development of spectrophotometric- and fluorometric-based bioanalytical assays, radiolabeled ligands are still commonly employed in pharmaceutical HTS applications. Although non-isotopic markers promise to be environmentally cleaner, safer, less expensive, and generally easier to use than radioactive compounds, sensitivity limitations have prevented these new methods from becoming widespread. Another major disadvantage of the competition assay is the number of steps, most notably washing steps, required to run assays.

Scintillation proximity assays, discussed for example in U.S. Patent No. 4,271,139 and U.S. Patent No. 4,382,074, were developed as a means of circumventing the wash steps required in the above heterogeneous assays. The homogeneous assay technology, which requires no separation of bound from free ligand, is based on the coating of scintillant beads with an acceptor molecule such as, for example, the target receptor.

In another approach to avoiding the use of radioactive labels, especially useful in high-throughput assays, lanthanide chelates are used in time-resolved fluorometry. *See, e.g.*, U.S. Patent No. 5,637,509.

Automated laboratory workstations have contributed significantly to advances in pharmaceutical drug discovery and genomic science. *See, e.g.*, U.S. Patent No. 5,104,621 and U.S. Patent No. 5,356,525. Particularly, robotics technology has played a major role in providing practical means for carrying out HTS methods. *See, e.g.*, U.S. Patent No. 4,965,049.

Robotic-based high-throughput tools are now routinely used for screening libraries of compounds for the purpose of identifying lead molecules for their therapeutic potential. For example, a screening method for characterizing ligand binding to a given target employing a

variety of separation techniques is described in WO 97/01755, and a related method is described in U.S. Patent No. 5,585,277.

Highly parallel and automated methods for DNA synthesis and sequencing have also contributed significantly to the success of the human genome project, and a competitive industry has developed. Examples of automated DNA analysis and synthesis include, *e.g.*, U.S. Patent No. 5,455,008; U.S. Patent No. 5,589,330; U.S. Patent No. 5,599,695; U.S. Patent No. 5,631,734; and U.S. Patent No. 5,202,231.

Computerized data handling and analysis systems have also emerged with the commercial availability of high-throughput instrumentation for numerous life sciences research and development applications. Commercial software, including database and data management software, has become routine in order to efficiently handle the large amount of data being generated.

With the developments outlined above in molecular and cellular biology, combined with advancements in combinatorial chemistry, there has been a huge increase in the number of targets and compounds available for screening. In addition, many new human genes and their expressed proteins are being identified by the human genome project and will therefore greatly expand the pool of new targets for drug discovery. A great need exists for the development of more efficient ultrahigh throughput methods and instrumentation for pharmaceutical and genomic science screening applications.

Miniaturization of chemical analysis systems, employing semiconductor processing methods, including photolithography and other wafer fabrication techniques borrowed from the microelectronics industry, has attracted increasing attention and has progressed rapidly. The so-called "lab-on-a-chip" technology enables sample preparation and analysis to be carried out on-board microfluidic-based cassettes. Moving fluids through a network of interconnecting enclosed microchannels of capillary dimensions is possible using electrokinetic transport methods.

Applications of microfluidics technology embodied in the form of analytical devices has many attractive features for pharmaceutical high throughput screening. Advantages of miniaturization include greatly increased throughput and reduced costs, in addition to low consumption of both samples and reagents and system portability. Implementation of these developments in microfluidics and laboratory automation hold great promise for contributing to advancements in life sciences research and development.

Of particular interest are microfluidics devices in which very small volumes of fluids are manipulated in microstructures, including microcavities and microchannels of capillary dimension, at least in part by application of electrical fields to induce electrokinetic flow of materials within the microstructures. Application of an electric potential between electrodes contacting liquid media contained within a microchannel having cross-sectional dimensions in the range from about 1 μm to upwards of about 1 mm results in movement of the contents within the channel by electroosmotic flow and/or by electrophoresis. Electrophoresis is movement of electrically charged particles, aggregates, molecules or ions in the liquid medium toward or away from the electrodes. Electroosmotic flow is bulk fluid flow, including movement of the liquid medium and of dissolved or suspended materials in the liquid medium. The extent of bulk fluid flow resulting from application of a given electrical field depends among other factors upon the viscosity of the medium and on the electrical charge on the wall of the microchannel. Both electroosmotic flow and electrophoresis can be used to transport substances from one point to another within microchannel device.

Electrophoresis has become an indispensable analytical tool of the biotechnology and other industries, as it is used extensively in a variety of applications, including separation, identification and preparation of pure samples of nucleic acids, proteins, and carbohydrates; identification of a particular analyte in a complex mixture; and the like. Of increasing interest in the broader field of electrophoresis is capillary electrophoresis ("CE"), where particular entities or species are moved through a medium in an electrophoretic chamber of capillary dimensions under the influence of an applied electric field. Benefits of CE include rapid run times, high separation efficiency, small sample volumes, *etc.* Although CE was originally carried out in capillary tubes, of increasing interest is the practice of using microchannels or trenches of capillary dimension on a planar substrate, known as microchannel electrophoresis ("MCE"). CE and MCE are increasingly finding use in a number of different applications in both basic research and industrial processes, including analytical, biomedical, pharmaceutical, environmental, molecular, biological, food and clinical applications.

Typically, the microchannels of MCE devices are constructed by forming troughs or grooves of appropriate dimension and configuration in one surface of a planar rectangular- or disc-shaped base substrate, and applying a planar cover to the surface to enclose the microchannels.

Conventionally, microchannels having capillary dimensions have been made in silicon or glass substrates by micromachining, or by employing photolithographic techniques. *See, e.g.*, U.S. Pat. No. 4,908,112, U.S. Pat. No. 5,250,263. Where the substrates are of fused silica, the microchannels can be enclosed by anodic bonding of a base and a cover. Exemplary MCE devices are also described in U.S. 5,126,022; U.S. 5,296,114; U.S. 5,180,480; and U.S. 5,132,012; and in Harrison *et al.*, "Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical Analysis System on a Chip," Science (1992) 261: 895; Jacobsen *et al.*, "Precolumn Reactions with Electrophoretic Analysis Integrated on a Microchip," Anal. Chem. (1994) 66: 2949; Effenhauser *et al.*, "High-Speed Separation of Antisense Oligonucleotides on a Micromachined Capillary Electrophoresis Device," Anal. Chem. (1994) 66:2949; and Woolley & Mathies, "Ultra-High-Speed DNA Fragment Separations Using Capillary Array Electrophoresis Chips," P.N.A.S. USA (1994) 91:11348.

Eckström *et al.* U.S. Pat. No. 5,376,252 describes a process for creating capillary size channels in plastic using elastomeric spacing layers. Öhman International Patent Publication WO 94/29400 describes a method for producing microchannel structures by applying a thin layer of a thermoplastic material to one or both of the surfaces to be joined, then joining the surfaces and heating the joined parts to melt the thermoplastic bonding layer. Kaltenbach *et al.* U.S. Pat. No. 5,500,071 describes constructing a miniaturized planar microcolumn liquid phase analytical device by laser ablating microstructures in the surface of a planar laser ablatable polymeric or ceramic substrate, rather than by conventional silicon micromachining or etching techniques.

U.S. Patent Application Serial No. 08/878,437 filed June 18, 1997 (Attorney Docket No. A-63519/RFT/BK SOAN-011) describes methods for fabricating microchannel structures constructed of a polymeric card-shaped or disc-shaped base plate having a planar surface in which a microchannel structure is formed, and a planar polymeric cover. The microchannel structure is enclosed by bonding the planar surfaces of the cover and the base plate together.

SUMMARY OF THE INVENTION

In one general aspect, the invention features a continuous form microstructure (*i.e.*, microcavity and/or microchannel) array device constructed as an elongate flexible film laminate containing a plurality of microstructures or arrays of microstructures arranged serially lengthwise along the laminate. Where the device has a series of microstructures, each structure

is configured to carry out a fluidic process or a step in a fluidic process. Where the device has a series of microchannel arrays, each array is configured to carry out a set of processes or steps, on an array of samples or of test reagents.

Because the microstructures, or arrays of microstructures, are serially arranged lengthwise along the laminate, the device can be fed lengthwise into and through an analytical device, and the structures or arrays can be treated serially in a continuous automated or semiautomated manner.

In some embodiments the flexible elongate laminate device is advanced within the analytic device from a continuous uncut supply roll, through the various parts of the analytical device and, as the laminate device is expended, to a takeup roll, similar to the way in which roll film is advanced frame-by-frame through a camera. In other embodiments the elongate laminate device is advanced within the analytic device from a continuous uncut accordion-folded supply stack, through the analytical device and, as the laminate device is expended, to an accordion-folded takeup stack. When the entire roll (or supply stack) has been expended and passed onto the takeup roll (or stack), the expended roll (or stack) can be discarded, or can conveniently and efficiently be stored in an archive, as may be desirable for some uses.

The microstructures are constructed either by forming channels, trenches or cavities of suitable dimension and configuration in a microchannel surface of a first lamina and, optionally, enclosing the channels by apposing a covering surface of a second lamina onto the microchannel surface to form the microstructures; by forming slits having suitable dimension and configuration in a spacing lamina, and sandwiching the spacing lamina between first and second enclosing laminae to enclose the slits between the apposed surfaces of the first and second enclosing laminae to form the microchannels or by combining a spacing lamina having slits therein with a lamina having such channels, trenches or cavities formed therein.

Electrodes can be formed in the device by any of a variety of techniques, known in the art, including application of wires or conductive decals, or printing or stamping using conductive inks, or vapor deposition, *etc.*, in a specific configuration onto a surface of one or both of the laminae. The laminate construction according to the invention is particularly suitable for application of flexible printed circuit technology. For technical review, *See*, Th. H. Stearns (1996), Flexible Printed Circuitry, SMTnet Bookstore. *See also*, U.S. 4,626,462; U.S. 4,675,786; U.S. 4,715,928; U.S. 4,812,213; U.S. 5,219,640; U.S. 5,615,088.

Processes for making flexible printed circuits are generally well known. Briefly, the electrodes, which provide connections from the reservoirs in the microfluidic structure to high-voltage contacts in an analytical device that carried the laminate, are formed within a thin polymer film laminate, which serves as a cover lamina to be affixed as described above to the base lamina, as described in more detail below.

In this context, an "analytical device" is a device that includes at least a detector capable of detecting or of measuring a signal produced in the course of the microfluidic process or process step, and means for moving the laminate in relation to the analytical device to bring a detection region in the microstructure within the field of the detector. Usually the analytical device is in a stable installation, and the laminate is advanced through it past the detector, but in some embodiments the laminate is held in place and the analytical device is moved along it. Of course, any number of such detectors may be employed, each alignable with a detection region (or series of detection regions, as the laminate progresses through). Usually, the analytical device also includes electrical contacts each alignable with a contact point in electrical circuitry employed to generate electroflow in the microstructure. Each such contact is electrically connected to a source of electrical power, and to control means (which may be automated) for changing the applied electric fields as the microfluidic process proceeds. The analytical device may further include means for adding various fluids (*e.g.*, samples, buffers or other solvents, reagents, and the like) to the microstructures by way of access ports in the laminate. The analytical device may additionally include means for changing the environmental conditions surrounding a portion of the laminate, such as temperature, and the like.

In some embodiments, the device is provided as an assembled laminate, in which the microchannels are fully enclosed; and in which ports or reservoirs are provided for introduction of sample or reagents or test compounds or liquid media; and in which electrodes have been emplaced and provided with leads for connection to a source of electrical power. Reagents, samples, test compounds, and/or media can be introduced as appropriate during or just prior to conducting the assays. In some embodiments the assembled laminate is provided with at least some of the media or reagents "on board" in the microchannels or reservoirs as appropriate. Where the device is provided with one or more substances already on board, the device can additionally be provided with means for protection of degradable contents from variations in ambient conditions and, particularly, for example, a release liner which resists loss

of moisture or of volatile contents and/or which resists light exposure to the contents, may be provided as a release liner on one or both surfaces of the laminate.

The device and method of the invention provides a full range of advantages in analytical sensitivity that inhere in the use of conventional microfluidic analysis, while at the same time providing for automated or semiautomated continuous processing of high numbers of analyses at high rates of speed. The complexity of mass screening programs, for example, is substantially reduced by elimination of many of the manipulation steps, whether by hand or by machine, that are required in use of conventional assay plates. And possibilities for error are reduced by reduction of the number of points at which manipulation by hand is required.

Methods and apparatus according to the invention for carrying out multiple microfluidic manipulations at high throughput rates are readily adaptable for automated non-contact dispensing of reagents or samples, providing for substantially reduced risk of cross-contamination.

Further, the continuous form assay array according to the invention significantly reduces the bulk volume of disposable materials, as compared with conventional assay card methods, both because the flexible laminates themselves are thinner than are conventional assay cards, and because the microchannel structures or arrays can be arranged on the continuous form device with more efficient use of the substrate surface area.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a diagrammatic sketch showing a portion of an embodiment of the laminate construction of a continuous form microchannel device of the invention.

Fig. 1B is a diagrammatic sketch of the portion of the embodiment of Fig. 1A, in an exploded view, showing the laminae.

Fig. 2A is a diagrammatic sketch of a portion of an alternate embodiment of the laminate construction of a continuous form microchannel device of the invention.

Fig. 2B is a diagrammatic sketch of the portion of the embodiment of Fig. 2A, in an exploded view, showing the laminae.

Figs. 3A, 3B are diagrammatic sketches in plan view of two alternative embodiments of microchannel structures configured as standard injection crosses, in which the separation channel is curved (Fig. 3A) or folded (Fig. 3B) to provide extended separation flow path length.

Fig. 3C is a diagrammatic sketch in plan view of an embodiment of a microchannel structure providing for introduction of four reagents into a sample flow path upstream from the separation channel, which is folded to provide extended separation flow path length.

Fig. 4 is a diagrammatic sketch in plan view of a portion of the length of an embodiment of a continuous form microchannel device of the invention, showing two in a series of microchannel arrays.

Fig. 5 is a diagrammatic sketch in a perspective view showing a method for constructing an elongate flexible film laminate having the general laminate structure shown in Fig. 1A.

Fig. 6 is a diagrammatic sketch in a side view showing a method for constructing an elongate flexible film laminate having the general laminate structure shown in Fig. 2A.

Figs. 7a, b are diagrammatic sketches in sectional view showing details of an embodiment of a device according to the invention made using a flexible circuit lamina.

Figs. 8a, b are diagrammatic sketches as in Figs. 7a, b showing details of an alternative embodiment of a device according to the invention made using a flexible circuit lamina.

Fig. 9 is a diagrammatic sketch showing a microstructure configuration that can be constructed in a continuous form laminate device of the invention, suitable for carrying out a receptor binding assay.

Fig. 10 is a diagrammatic sketch showing a microstructure configuration that can be constructed in a continuous form laminate device of the invention, suitable for carrying out an enzyme assay.

Fig. 11a is a diagrammatic sketch of a portion of the length of an embodiment of a base lamina of a continuous form microstructure device of the invention, showing two in a series of microchannel arrays. Each microchannel array includes four microstructures each configured to carry out a receptor binding assay, as described with reference to Fig. 9.

Fig. 11b is a diagrammatic sketch of a portion of the length of flexible circuit laminate showing two in a series of layouts of electrodes and electrical contacts, each layout configured to serve a microchannel array as shown in Fig. 11a.

Fig. 11c is a diagrammatic sketch of a portion of the length of an embodiment of a continuous form elongate laminate microstructure device of the invention, constructed by laminating the flexible circuit laminate of Fig. 11b onto the base lamina of Fig. 11a.

Fig. 12 is an isometric view of a laminate microstructure device of the present invention and a contact probe assembly for use therewith.

Fig. 13 is a cross-sectional view of the laminate microstructure device of Fig. 12 taken along the line 13-13 of Fig. 12 with another embodiment of a contact probe assembly for use therewith.

Fig. 14 is a cross-sectional view similar to Fig. 13 of another embodiment of a laminate microstructure device of the present invention and another embodiment of a contact probe assembly for use therewith.

Fig. 15 is a plan view of a further embodiment of a laminate microstructure device of the present invention.

Fig. 16 is a cross-sectional view of the laminate microstructure device of Fig. 15 taken along the line 16-16 of Fig. 15.

Fig. 17 is a cross-sectional view similar to Fig. 13 of yet another embodiment of a laminate microstructure device of the present invention and an contact\ probe for use therewith.

Fig. 18 is a top plan view, partially cut away, of another embodiment of a laminate microstructure device of the present invention.

Fig. 19 is a cross-sectional view of the laminate microstructure of Fig. 18 taken along the line of 19-19 of Fig. 18.

The drawings are diagrammatic only and not to scale and, particularly, in some of the Figs. the thicknesses of the laminate composites and of the layers of which they are constructed are much exaggerated for clarity of presentation.

DETAILED DESCRIPTION

Construction

In General

"Microfluidic processing", as that term is used herein, means and refers to fluid processing—that is, fluid handling, transport and manipulation—carried out within chambers and channels of capillary dimension. Valveless sample injection is achieved by moving fluid from reagent reservoirs into cross-channel injection zones, where plugs of buffer or test compounds are precisely metered and dispensed into a desired flowpath. The rate and timing of movement of the fluids in the various microchannels can be controlled by electrokinetic,

magnetic, pneumatic, and/or thermal-gradient driven transport, among others. These sample manipulation methods enable the profile and volume of the fluid plug to be controlled over a range of sizes with high reproducibility. In addition, microfluidic processing includes sample preparation and isolation where enrichment microchannels containing separation media are employed for target capture and purification. Microfluidic processing also includes reagent mixing, reaction/incubation, separations and sample detection and analyses.

Generally, the expression "microstructure", as used herein, means and refers to a single enclosed microchannel or a network of interconnecting microchannels having cross-sectional dimensions suitable for carrying out microfluidic manipulations of materials carried by them. Several steps or stages of an analytical process may be carried out in one microchannel structure, suitably configured. Configurations of various complexity are disclosed for example in U.S. Patent Application Ser. No. 08/902,855 filed July 30, 1997 [Attorney Docket No. A-62855-1/RFT/BK SOAN-8-1] and in U.S. Patent Application Ser. No. 08/878,447 filed June 18, 1997 [A-64739/RFT/BK SOAN-017], the entire contents of each of which are incorporated herein by this reference.

A "microfluidic network", as that term is used herein, is a system of interconnected microchannels, *i.e.*, cavity structures and capillary-size channels, through which fluids can be manipulated and processed.

Cavity structures, in the context of microstructures, are spaces, usually formed in, *e.g.*, a planar substrate, a plate, or the like in accordance with the present invention. Cavity structures include, *e.g.*, wells, reservoirs, chambers for incubation or separation or detection, and the like. Cavity structures can be present at one or both of the termini, *i.e.*, either end, of a channel, and are there usually referred to as reservoirs. Such cavities structures may serve a variety of purposes, such as, for example, means for introducing a buffer solution, elution solvent, reagent rinse and wash solutions, and so forth into a main channel or one or more interconnected auxiliary channels, receiving waste fluid from the main channel, and the like. In some embodiments, cavity structures are not connected by channels, but rather stand alone; such free standing cavities can be used for reagent introduction, on-board mixing, incubation, reactions, detection and the like. In another embodiment, these individual steps of a homogeneous assay can be carried out in a cavity.

In the microstructures of the invention "channels", usually "microchannels", provide conduits or means of communication (usually fluid communication and more particularly liquid

communication), between cavity structures and the like. Channels include capillaries, grooves, trenches, microflumes, and so forth. The channels may be straight, curved, serpentine, labyrinth-like or other convenient configuration within the planar substrate. The cross-sectional shape of the channel may be circular, ellipsoidal, trapezoidal, square, rectangular, triangular and the like within the planar substrate in which it is present.

The inside of the channel may be coated with a material to improve the strength of the microstructure, for modifying, enhancing or reducing electroosmotic flow, for enhancing or reducing electrophoretic flow, for modification of surface hydrophobicity/hydrophilicity, for binding of selected compounds, and so forth. Exemplary coatings are silylation, polyacrylamine (vinyl-bound), methylcellulose, polyether, polyvinylpyrrolidone, and polyethylene glycol, polypropylene, Teflon™ (DuPont), Nafion™ (DuPont), polystyrene sulfonate and the like may also be used. *See also* U.S. Patent Application Serial No. 08/715,338, the relevant disclosure of which is incorporated herein by reference.

A "microchannel", as that term is used herein, is an at least partly enclosed trench or channel or cavity having capillary dimensions, that is, having cross-sectional dimensions that provide for capillary flow along the channel. Usually at least one of the cross-sectional dimensions, *e.g.*, width, height, diameter, is at least about 1 μm, usually at least 10 μm; and is usually no more than 500 μm, preferably no more than 200 μm. Channels of capillary dimension typically have an inside bore diameter ("ID") of from about 10 to 200 microns, more typically from about 25 to 100 microns.

Microchannels can provide for electroflow between cavity structures and the like in the microstructures of the invention. "Electroflow", as used herein, is the manipulation of entities such as molecules, particles, cells, vitreous fluid and the like through a medium under the influence of an applied electric field by use of electrodes and the like to induce movement such as electrokinetic flow, electroosmotic flow, electrophoretic flow, dielectrophoretic flow, and so forth. Depending upon the nature of the entities, *e.g.*, whether or not they carry an electrical charge, as well as upon the surface chemistry of the chamber in which the electroflow is conducted, the entities may be moved through the medium under the direct influence of the applied electric field or as a result of bulk fluid flow through the pathway resulting from the application of the electric field, *e.g.*, electroosmotic flow. It is within the purview of the present invention that electroflow can be carried out in conjunction with movement of material by other means than application of an electric field, such as by gravity or by application of a

magnetic field, centrifugal force, thermal gradients, aspiration, negative pressure, pumping, pneumatic forces, and the like.

An "electroflow medium" is an electrically conductive medium, that is generally utilized in carrying out microfluidic processes. The particular medium chosen is one that is suitable to a particular application of the present invention. Such media include, for example, buffer solutions, cross-linked and uncross-linked polymeric solutions, organic solvents, detergents, surfactant micellular dispersions, gels of the type generally used in connection with analytical separation techniques and other microfluidic processes, and so forth. For example, cross-linked polyacrylamide gel, cellulose derivatives, uncross-linked polyacrylamide and derivatives thereof, polyvinyl alcohols, polyethylene oxides and the like may be used. For a discussion of such media *see, e.g.*, Barron and Blanch, "DNA Separations by Slab Gel and Capillary Electrophoresis: Theory and Practice", Separation and Purification Methods (1995) 24:1-118.

Suitable electroflow media include conventional buffers such as, for example, the Good's buffers (HEPES, MOPS, MES, Tricine, etc.), and other organic buffers (Tris, acetate, citrate, and formate), including standard inorganic compounds (phosphate, borate, etc.). Exemplary buffer systems include: (i) 100 mM sodium phosphate, pH 7.2; (ii) 89.5 mM tris-base, 89:5 mM Boric acid, 2 mM ETDA, pH 8.3. Buffer additives include: methanol, metal ions, urea, surfactants, and zwitterions, intercalating dyes and other labeling reagents. Polymers can be added to create a sieving buffer for the differential separation of molecular species, such as, *e.g.*, nucleic acids, proteins, and the like, based on molecular size. Examples of such polymers are: polyacrylamide (cross-linked or linear), agarose, methylcellulose and derivatives, dextrans, and polyethylene glycol. Inert polymers can be added to the separation buffer to stabilize the separation matrix against factors such as convective mixing.

Alternatively, buffers containing micelles can be used for effecting separation of electrically neutral or hydrophobic substances of interest. The micelles are formed in the buffer by addition of an appropriate surfactant at a concentration exceeding the critical micelle concentration of that detergent. Useful surfactants include but are not limited to sodium dodecyl sulfate, dodecyltrimethyl ammonium bromide, *etc.* Weakly charged or apolar analytes partition into the micelles to different degrees depending upon their degree of hydrophobicity and thus can be separated. This subtechnique of capillary electrophoresis is termed micellar electrokinetic chromatography.

"Electrophoresis" is separation of components in a liquid by electroflow. Various forms of electrophoresis include, by way of example and not limitation, free zone electrophoresis, gel electrophoresis, isotachophoresis, high performance CE, capillary zone electrophoresis, and the like. In the context of the microstructures according to the invention, an "electrophoresis column" is a channel for carrying out electrophoresis.

A microstructure can be made by forming one or more trenches or channels or cavities in the desired configuration and with the desired dimensions in one surface of a lamina, and then optionally covering selected portions at least of the trenches or channels or cavities with a second lamina to form one or more enclosed microchannels. Or, a microstructure can be made by forming slits in the desired configuration and with the desired dimensions through a spacing lamina having a desired thickness, and then enclosing selected portions at least of the slits by sandwiching the spacing lamina between two enclosing laminae to form one or more enclosed microchannels.

As noted above, the enclosed volumes within the microchannels provide "flow paths", in which the various components of the analytical process can move and combine and interact or react, and in which analytes can be separated electrophoretically or retained by capture media. Any of a variety of means can be employed to provide sources of supply of the various components to the flow paths.

Any of a variety of means can be employed to cause movement of the various components within the microchannels. Usually, as noted above, an electric field is applied to a segment of a microchannel to cause electrokinetic transport (by electroosmotic flow or by electrophoresis, or by some combination of EOF and electrophoresis) of the contents of the microchannel segment. An electric field can be applied by positioning a pair of electrodes, connected to a source of electrical power, within the microchannel at the ends of the microchannel segment. Where it is desired, for example, to move a buffer from a buffer reservoir along a microchannel to a buffer waste reservoir, the pair of electrodes can be positioned so that they contact the fluid within the respective reservoirs; application of an electric potential across the electrodes induces a electrokinetic flow from one reservoir to the other through the microchannel.

Additionally, as noted above, other means than electrokinetic flow may be used to move the components within the microchannels, and, particularly, to fill the microchannel structure

at the outset, or to introduce an aliquot of sample material or of a test compound, for example, at the beginning of or in the course of the analysis.

As used herein, the expression "array of microchannel structures" means and refers to a set of microchannel structures, typically but not necessarily all having the same or similar configurations, each operating to carry out one of a set of related analyses, as will be described more fully below. A microstructure or an array of microstructures can according to the invention be arranged within the laminate structure so that the positions of various of the cavities correspond to particular useful sites in conventional sample holding or sample delivery apparatus. Thus, for example, certain of the cavities may be arranged and spaced apart to correspond to the dimensions and configurations of a standard multiwell plate, which has an array of wells. Standard plates may have any number of wells, usually in a pattern, and usually numbering 96, 192, 384 or 1536 wells or more. Examples of such multiwell plates are microtiter plates having a pattern of wells. The wells extend into the substrate forming the plate, and are open at the top surface of the plate and closed at the bottom. There are no openings, holes or other exits from the wells other than from the top surface at the opening of the well. Similarly, a transfer plate may have a like arrangement of apertures or nozzles, and at least selected ones of the cavities in the microstructure or microstructure array according to the invention can accordingly be arranged so that direct transfer can be made from the plate to the microcavity network.

Other arrangements for the arrays of microchannel structures are possible, according to the particular dispensing requirements, among other factors. For example, an array of 96 microstructures may be in a 12 × 8 orthogonal arrangement, corresponding to the positions of wells in a 96-well microtiter plate; or in a linear arrangement of 96 microstructures, or any other arrangement. And, an array of 384 microstructures may be in a 24 × 16 orthogonal arrangement, corresponding to the positions of wells in a 384-well microtiter plate; or in a linear arrangement of 384 microstructures, or any other arrangement.

Depending upon the type of analysis to be performed, any of various liquid media including buffers or solvents or electrophoretic separation media, reagents, *etc.*, may be brought into play in the course of the analysis.

At one or more points in the analytical process, detection and/or measurement of one or more analytes is required. The analyte or analytes may be, for example, a plurality of electrophoretically resolved reaction products, such as restriction fragments of a nucleic acid,

bound and free fractions in a ligand-binding assay, substrate and product of an enzymatic reaction, and the like.

The Laminate

Referring now to Figs. 1A, 1B, there is shown at 10 a portion of an embodiment of an elongate flexible film laminate or microstructure device according to the invention, as assembled (Fig. 1A) and in an exploded view in which the laminae appear as separated (Fig. 1B). In Figs. 1A and 1B, as in Figs. 2A, 2B, only a short segment of the full length of the laminate is shown, as suggested by broken lines indicating that the laminate extends lengthwise beyond the margins of the drawing. In the embodiment of Figs. 1A, 1B, the microchannel structure is formed in a spacing lamina 11 sandwiched between a base lamina 12 and a cover lamina 14. Slits 16 having capillary cross-sectional dimensions are formed through spacing lamina 11, and are enclosed by apposed surfaces 13, 15 of base lamina 12 and cover lamina 14 in the composite structure. Fig. 1B shows slits forming walls of just two 17, 19 of many microchannel structures serially arranged lengthwise on the elongate laminate. In the example shown in Figs. 1A, 1B, each microchannel structure has a simple cross configuration formed by enclosure of a pair of intersecting slits.

As will be appreciated, the widths of the microchannels resulting from the construction illustrated in Figs. 1A, 1B is established by the width of the slits in the spacing lamina; and the thickness of the microchannels is established by the distance between the apposed surfaces 13, 15 of the enclosing laminae 12 and 14, which approximates the thickness of the spacing layer. As noted above, the microchannels are of capillary dimension, that is, the larger cross-sectional dimension (usually the width) of the microchannel is usually no greater than about 750 μm, more usually no greater than about 500 μm, and most usually in the range from about 100 μm to about 250 μm; and the smaller cross-sectional dimension (usually the depth) can be somewhat smaller.

Usually, as noted generally above, reservoirs or access ports or receptacles are provided for introducing the various components of the analytic process (sample, buffers or solvents, test compounds, *etc.*) into the microchannel structures. These can be in the form, for example, of perforations 9 through the base lamina 12 or through the cover lamina 14, as illustrated in Fig. 1B. Where, as shown for example in Fig. 1B, the reservoirs or access ports or receptacles are formed in a lamina other than the one in which the channels are formed, they must be located so as to be suitably aligned with appropriate sites in the microchannel structure when

the composite is assembled. Accordingly, in Fig. 1B, the perforations 9 in the cover lamina 12 are arranged to be aligned with the ends of the microchannels formed in the spacing layer 11 when the spacing lamina 11 is sandwiched between the apposed surfaces 13, 15 of the base lamina 12 and the cover lamina 14.

To provide for predictable and consistent microfluidic movement, mixing, and separations, the microchannels in the laminate composite device must be adequately dimensionally stable, and the apposing surfaces 13, 15 of the enclosing laminae 12, 14 must be adequately sealed to the surfaces of spacing lamina 11, at least at the margins of the slits, to keep the fluids within the flow paths formed by the microchannels from escaping between the laminae. These requirements are met by appropriate selection of materials and thicknesses of the films making up the laminae, and by appropriate selection of means for sealing the contact surfaces of the laminae.

As noted above, each of the laminae is a flexible film, usually firm enough to hold the shape and dimensions of the microchannels, yet sufficiently compliant to provide a desired flexibility in the composite laminate device. Preferred films include acrylics and polyethylenes, for example. Preferred means for sealing will be selected according to the film materials in the laminae to be joined. Particularly, for example, the film materials and adhesives described in USSN 08/878,437 filed June 18, 1997 (Attorney Docket No. A-63519/RFT/BK SOAN-011), the disclosure of which is hereby incorporated herein in its entirety.

In the embodiment of Figs. 1A, 1B, the thickness of the spacing lamina is selected to provide the desired microchannel depth, taking into account any effect (additive or subtractive) that the sealing process may have on the distance between the apposed surfaces 13, 15 of the enclosing laminae.

In addition to the spacing lamina 11 and the enclosing laminae 12, 14, the laminate may further include release liners 16 and/or 18. Use of a release liner may be especially desirable where at least some of the components of the analytical process (a reagent or a buffer, for example) are provided on board the device prior to use. Such release liners can mitigate degradation or loss of the contents of the device during prolonged exposure to varying environmental conditions that may be encountered prior to use of the device, as for example during storage. It may be particularly important, for example, to avoid loss or intrusion of moisture or of more volatile substances out from or into the microchannel structure. Or, it may be important to avoid exposure to light. Accordingly, preferred release liners form a barrier to

movement of moisture or volatile materials, and thin polymer films, including metallized films may be particularly suitable.

Referring now to Figs. 2A, 2B, there is shown at 20 a portion of an alternate embodiment of an elongate flexible film laminate or microstructure device according to the invention, a assembled (Fig. 2A) and in an exploded view in which the laminae appear as separated (Fig. 2B). In this embodiment, the microchannel structures 27,29 are constructed by forming channels or trenches 26 in a surface 21 of base lamina 22, and apposing a surface 15 of a cover lamina 14 onto surface 21 to enclose the microchannels. Reservoirs or access ports or receptacles can be provided for introduction of process components into and/or for removal of excess or waste from the microchannel structure, as noted with reference to Figs. 1A, 1B. These are illustrated by way of example in Fig. 2A as perforations 9 through cover lamina 14, positioned so as to be suitably aligned with the channels or trenches 26 in the base lamina 22 when the surfaces 21, 15 of base lamina 22 and cover lamina 14 are apposed.

Alternatively, reservoirs may be provided in base lamina 22, in the form of wells or holes through the thickness of base lamina 22, each situated in fluid communication with a microchannel or trench, as may be desired. And, referring again to Figs. 1A, 1B, reservoirs may be provided in the spacing lamina 11, each in fluid communication with a slit. If the base lamina 22 (or the spacing lamina 11) is sufficiently thick, reservoirs of significantly high volume can be provided in this way, and the cover lamina (or enclosing laminae) can be very thin. For reservoirs that are loaded in the course of the lamination process, no access opening through either the cover lamina or the opposite surface of the base lamina (or either of the spacing laminae) is required; however, for any such reservoirs that are to be loaded after the laminate has been formed, access openings aligned with the reservoirs can be provided, for example as holes through the cover lamina or through the base lamina (or through a spacing lamina).

In this embodiment the widths and depths of the microchannels are established by the dimensions of the trenches or channels formed in the base lamina. Accordingly, precise control of the dimensions during the formation of the trenches or channels, taking account of any additive or subtractive effect of the sealing process, results in reproducible microchannel dimensions.

As in the embodiment of Figs. 1A, 1B, the embodiment of Figs. 2A, 2B may additionally include release liners 16 and/or 18.

As in the embodiment of Figs. 1A, 1B, each of the laminae in the embodiment of Figs. 2A, 2B is a flexible film. Preferred film materials for the base lamina 22 and cover lamina 14 are polymer films; and preferred sealing means are selected according to the film materials to be joined. The base lamina 12 preferably is sufficiently thick to maintain its structural integrity after the trenches or channels have been formed in it. Particularly, for example, where the configuration of the microchannel structure is complex, or where there is a high density of trenches or channels, the mechanical strength of the base lamina may be compromised, and for ease of handling as well as to maintain the dimensionality of the microchannel structure during assembly and use, the base lamina should be thick enough so that it maintains its mechanical integrity.

Detection is usually optical, and most usually the signal is generated by laser-induced fluorescence; the detector is usually a conventional confocal optical system. Other detections means may be employed.

As noted above, each of the microchannel structures shown in Figs. 1B, 2B is configured as a simple injection cross, formed by intersection of two straight microchannels. Such a configuration is useful, for example, in carrying out a quantitative electrophoretic separation of a metered sample volume, as described for example in U.S. Patent Application Ser. No. 08/878,447 filed June 18, 1997 [SOAN-017]. The intersecting microchannels of a simple injection cross need not be straight, and in some configurations more efficient use of the substrate area is made possible by configuring one or more microchannel arms otherwise. Referring now for example to Figs. 3A, 3B, alternative embodiments of simple injection cross configurations are shown in which one electrophoretic microchannel is made relatively longer. In each of microchannel structure configurations or microstructures 30, 32, a shorter microchannel and a longer microchannel intersect at 31 to form an injection cross. Sample supply reservoir 36, sample drain reservoir 37, elution buffer reservoir 33, and analyte waste reservoir 34 are provided at the ends of the microchannel segments; and an electrode (not shown in the Figs.) connected to a source of electrical energy is positioned to contact the liquid contained within each reservoir. Potential differences across the electrodes are adjusted first to draw the sample electrokinetically from sample supply reservoir 36 across intersection 31 toward sample drain reservoir 37; and then to draw a metered volume of sample from intersection 31 into separation channel 35. As the sample plug proceed electrokinetically through separation channel 35 toward analyte waste reservoir 34, the sample becomes

electrophoretically separated into its analyte components, which are detected at a downstream detection region point in separation channel 35. As will be apparent in the Figs., the electrophoretic separation channel is made relatively much longer by forming it as a spiral turning one or more times around intersection 31 and reservoirs 33, 36, 37, and the shorter microchannel arms (Fig. 3A), or by forming it in a folded configuration (Fig. 3B). The resulting microchannel structures occupy a compact area of the substrate, and can be particulay useful in microchannel arrays, as will be described more fully below with reference to Fig. 4.

The microchannel structures can be formed in more complex configurations, according to the analytical process to be carreid out in them. Referring now to Fig. 3C, there is shown by way of example at 38 a microchannel structure or microstructure having an intersection 31 forming an injection cross, and having sample supply reservoir 36, sample rain reservoir 37, elution buffer reservoir 33, and extended electrophoretic separation channel 35 leading to waste reservoir 34. In this embodiment, microchannels enclosing flow paths running from four additional supply reservoirs 39 to four additional downstream drains 40 additionally cross the microchannel downstream from the intersection 31. These additional flow paths provide for sequential introduction of four additional analytical components (which may be reagents, or test compounds, or buffers, *etc.*) to the moving sample plug.

An example of a microchannel array is shown in a plan view in Fig. 4, illustrating a way in which the arrangement of the microchannels structures in the array can be made to match the geometry of, for example, a standard 96-well plate. Such an arrangement can facilitate automated transfer of samples or of test compounds from the standard plate to the continuous form microchannel device of the invention, providing for efficient transfer with reduced waste and minimal cross-contamination. Fig. 4, for example, shows a short segment of an elongate flexible film laminate containing a series of microchannel arrays according to the invention. The elongate flexible film laminate 42 extends lengthwise beyond the range of the drawing, as indicated by broken lines extending from the edges 41 of the short segment. The short segment shown, which is limited by lines 43, includes two successive microchannel arrays or microstructures 44, 45. Each of the microchannel arrays 44, 45 in this example contains 96 microchannel structures 30, each configured as in the example shown in Fig. 3A, and all arranged in an orthogonal 12 × 8 grid that conforms to the geometry of a conventional 96-well plate.

Manufacture

The basic technique and machinery for bringing the laminae together to form the laminate composite according to the invention are generally known, and, depending upon the materials that make up the various laminae, any of a variety of film lamination techniques can be used.

Figs. 5 and 6 are sketches showing in general outline schemes for constructing the laminate embodiments of Figs. 1A and 2A. Referring now to Fig. 5, there are shown rollers 51, 52, and 54, carrying film materials to serve as, respectively, a spacing lamina 11, a base lamina 12, and a cover lamina 14. Slits 16 may be cut through spacing lamina 11 before it is rolled onto roller 51, so that the spacing lamina comes off roller 51 with the configuration of the microchannel structures already in place; or, as illustrated in Fig. 5, a cutting tool 57 may operate to cut the slits in the predetermined pattern as spacing lamina 11 is drawn from roller 51. Similarly, access openings or reservoirs 9 can be formed by perforating base lamina 12 or (as in Fig. 5) cover lamina 14 before it is stored on roller 54, so that during assembly the cover lamina comes off roller 54 with the perforations already in place; or, as illustrated in Fig. 5, a cutting tool 59 may operate to cut the predetermined pattern of perforations as cover lamina 14 is drawn from roller 54. In either method, preferred tools for cutting slits and perforations include lasers (laser cutting or laser ablation) and die cutting, for example.

Laminae 11, 12, and 14 are apposed by drawing them between rollers 53. As will be appreciated, it is essential that the perforated enclosing lamina be appropriately aligned with the spacing lamina during the lamination process, so that the perforations will be suitably aligned with the microchannels in the assembled device. Any registration technique may be used to ensure proper alignment in the longitudinal direction. Preferably, sprocket holes can be cut in one or both margins of the laminae, and the respective sprocket holes can be aligned on a sprocket. It can be suitable to provide a sprocket drive at the rollers 53, for example.

As noted generally above, certain of the components of the analytic process to be carried out in the device (buffer or solvent, separation media, *etc.*) can be loaded into portions of the microchannel structure before use. Particularly, it may be desirable to load certain of the constituents before enclosing the microchannels. This may be true, for example, if one or more constituents has a high viscosity at ambient temperatures, as may be true of certain electrophoretic separation media. Accordingly, as illustrated in Fig. 5, the assembled laminate formed of the spacing layer 11 enclosed by base layer 12 and cover layer 14 is drawn through

a filling workstation 69, by conventional tractor means, where the selected components are injected or drawn by suction into the appropriate microchannels by way of the access perforations.

And, as noted above, where one or more components are provided on board the device, it maybe desirable to seal one or both surfaces of the device with release liners. Accordingly it is optional, as shown in Fig. 5, as the assembled and filled laminate is drawn toward takeup roller 55, to draw release liners 16 and 18 from rollers 66, 68 and between rollers 56, to appose the release liners onto the surfaces of the enclosing laminae 12 and 14. Alternatively, where the nonperforated enclosing layer is impermeable to the contents of the assembled and filled microchannel laminate of spacing layer 11 and enclosing layers 12, 14, sufficient protection of the contents can be provided by the contact of the nonperforated surface and the perforated surface when the device is rolled onto takeup roller 55, on which the device can be stored for use.

Similarly, referring now to Fig. 6, there are shown rollers 64, 62, carrying film materials to serve as, respectively, a cover lamina 14 and a base lamina 22. Channels or trenches 26 may be formed in surface 21 of base lamina 22 before it is rolled onto roller 62, so that the base lamina comes off roller 62 with the configuration of the microchannels already in place; or, as illustrated in Fig. 6, a cutting tool (or other means, as described in more detail below with reference to Figs. 7 through 9) 67 may operate to form the trenches or channels in the predetermined pattern as base lamina 22 is drawn from roller 62. Suitable cutting techniques employ, for example, controlled laser ablation, using equipment and techniques well known in the laser micromachining industry. Suitable laser micromachining systems and protocols for their use are available from, for example, Resonetics, Nashua, NH.

Other means for forming channels, cavities or trenches include but are not limited to heat embossing, hot embossing, ultraviolet embossing, ultraviolet curing of a liquid substance, patterning a thin film which extruding or hot stamping a surface of a film layer prior to lamination. Known micromachining techniques including. *e.g.*, photolithographic techniques, may also be employed in forming the microstructures in the film surfaces. Alternative methods also include ultrasonic forming, pressure forming and thermal forming, vacuum forming, blow molding, stretch molding, insert molding, injection molding, extrusion casting, compression molding, encapsulation processes, thermoforming and digital printing, any of which may be

employed in a continuous-form process according to the invention. Any suitable techniques such as are known in the plastics micromachining art may be employed.

Similarly, access openings or reservoirs 9 can be formed by perforating cover lamina 14 before it is stored on roller 64, so that during assembly the cover lamina comes off roller 64 with the perforations already in place; or, as illustrated in Fig. 6, a cutting tool 59 may operate to cut the predetermined pattern of perforations as cover lamina 14 is drawn from roller 64. In either method, preferred tools for perforating the cover lamina include lasers and die cutters, for example, as described above with reference to Fig. 5, for example.

Laminae 14 and 22 are apposed by drawing them between rollers 63, and properly aligned as described above with reference to Fig. 5.

Here, as in the embodiment of Fig. 5, the assembled device can be provided with one or more of the analytical components on board. Components can be loaded into the assembled device by drawing the assembled laminate formed of the base layer 22 and the cover layer 14 through a filling workstation 69, as described above with reference to Fig. 5. And, optionally where desired, as the assembled and filled laminate is drawn toward takeup roller 65, release liners 16 and 18 may be drawn from rollers 66, 68, and between rollers 56, to appose the release liners onto the surfaces of the laminate for protection.

In some embodiments according to the invention, the reservoir and microchannel are formed in the base lamina, and the flexible circuit laminate forms a cover lamina. In one approach, illustrated in Figs. 7a and 7b, the flexible circuit laminate (that is, the cover lamina) is made up of two layers, namely, a seal layer and a back layer. In this embodiment part of the conductive trace is formed on the back surface of the seal layer, and part is formed in the front surface of the back layer. In another approach, illustrated in Figs. 8a and 8b, the flexible circuit layer is made up of three layers, namely a seal layer, which carries no conductive trace, and two circuit layers, each carrying a conductive trace. One of these circuit layers is a back layer, and the other is laminated between the back layer and the seal layer.

Referring now to Figs. 7a, 7b, there is shown generally at 70 a portion of a microstructure device according to the invention, in transverse section thru a reservoir and microchannel and associated circuitry. The device consists of a base lamina 72, constructed of a generally planar plastic material 74, a seal layer 76, formed of a low fluorescence polymer film 77, and a back layer 78, formed of a plastic film 79. Formed in the polymer base lamina 74 are reservoir or well 71 and microchannel 73 of a microstructure. An opening 75 is formed

through the seal layer film 77 in register with the reservoir 71. A front surface of seal layer film 77 is provided with an adhesive 82, which will serve to seal the seal layer and the base layer together when assembled, as shown in Fig. 7b. A rear surface of the seal layer is provided with contact conductive trace portion or trace 83 of the circuitry. A detection clearance opening 80 is formed through back layer film 79 in register with a detection zone of the microchannel 73, and a contact opening 81 is formed through back layer film 79 in register with the contact conductive trace portion 83. A front surface of the back layer film 79 is provided with a second conductive trace 85, having one region in register with a region of the contact conductive trace 83 and another region in contact with a carbon electrode or electrode portion 86, which in turn is in register with the reservoir 71. A conductive adhesive 84 provides for good conductive adhesion between conductive traces 83, 85, when assembled, as shown in Fig. 7b. It should be appreciated that layers 72, 76 and 78 can optionally be sandwiched between top and bottom release layers (not shown) similar to layers or liners 16,18 discussed above. The top release layer can seal reservoir 71. The bottom release layer can be provided with openings in registration with openings 80,81 in the back layer 78. Referring now to Fig. 7b, an electrical contact or electrode probe 88 in the analytical instrument contacts the conductive trace portion or contact portion of the circuitry by way of the contact opening in the back layer, and a photodetector (not shown in the Figs.) detects the signal in the microchannel through the low fluorescence film of the seal layer by way of the detection opening 80 in the back layer. Conductive traces 83,85 and carbon electrode 86 are included in the electrical means of microstructure device 70.

Where laser-induced fluorescence detection is employed, preferred low fluorescence materials have sufficiently low fluorescence at the illuminating and back scattering wavelengths that the presence of the film in the optical path does not significantly reduce detection. Examples of suitable such materials include impact modified acrylic (*e.g.*, Rohm film 99530), polyethylene terephthalate ("PET"), polyolefins (*e.g.*, Zeonex), and acetates. The adhesive also preferably has low fluorescence characteristics, and preferably has surface characteristics similar to those of the walls of the channel, inasmuch as the adhesive will form one inner surface of the microchannel when assembled, and differences could a adversely affect electroflow in the channel. Suitable such adhesives include organic based acrylic adhesives.

Referring now to Figs. 8a, 8b, there is shown generally at 170 a portion of an alternative embodiment of a microstructure device according to the invention, in transverse section thru a reservoir and microchannel and associated circuitry. The device consists of a base lamina 172, constructed of a generally planar plastic material 174, a seal layer 176, formed of a low fluorescence polymer film 177, a back circuit layer 178, formed of a plastic film 179, and an intermediate circuit layer 190, formed of a polymer film 191. Formed in the polymer base lamina 174 are reservoir or well 171 and microchannel 173 of a microstructure. An opening 175 is formed through the seal layer film 177 in register with the reservoir 171. A front surface of seal layer film 177 is provided with an adhesive 182, which will serve to seal the seal layer and the base layer together when assembled, as shown in Fig. 8b. A back surface of the intermediate circuit layer film 191 is provided with contact conductive trace portion or trace 183 of the circuitry, and a front surface of the intermediate circuit layer film 191 is provided with an adhesive 192, which will serve to seal the intermediate circuit layer film 191 to the seal layer 177 when assembled, as shown in Fig. 8b. An opening 195 is formed through the intermediate circuit layer 190, in register with the opening 175 in the seal layer and with the reservoir 171. An intermediate detection clearance opening 193 is formed through intermediate circuit layer film 191 in register with a detection zone of the microchannel 173. A detection clearance opening 180 is formed through back layer film 179 in register with a detection zone of the microchannel 173, and a contact opening 181 is formed through back layer film 179 in register with the contact conductive trace portion 183. A front surface of the back layer film 179 is provided with a second conductive trace 185, having one region in register with a region of the contact conductive trace 183 and another region in contact with a carbon electrode or electrode portion 186, which in turn is in register with the reservoir 171. A conductive adhesive 189 provides for good conductive adhesion between conductive traces 183, 185, when assembled, as shown in Fig. 8b. It should be appreciated that layers 172, 176, 178 and 190 can optionally be sandwiched between top and bottom release layers (not shown) similar to layers or liners 16,18 discussed above. The top release layer can seal reservoir 171. The bottom release layer can be provided with openings in registration with openings 180,181 in the back layer 178. Referring now to Fig. 8b, an electrical contact or electrode probe 188 in the analytical instrument contacts the contact conductive trace portion or contact portion of the circuitry by way of the contact opening in the back layer, and a photodetector (not shown in the Figs.) detects the signal in the microchannel through the low fluorescence film of the

seal layer by way of the detection opening 180 in the back layer. Conductive traces 183,185 and carbon electrode 186 are included in the electrical means of microstructure device 170. In this embodiment, the flexible circuit laminate (made up of the two circuit layers) can be constructed separately from the base layer and seal layer, because the seal layer does not include any circuitry. Moreover, because in this embodiment there need not be a good seal between the flexible circuit laminate and the microchannels in the base layer, it is not necessary that the flexible circuit laminate have a surface that conforms precisely with the surface of the base layer.

An embodiment of a microstructure array device according to the invention, provided with flexible circuitry constructed generally as described above, is shown in Figs. 11a, 11b, 11c. In this example, the elongate flexible film laminate contains a plurality of microstructure arrays arranged serially lengthwise along the laminate. Each microstructure array in this illustrative embodiment includes four microstructures, each configured to carry out an analytic process.

Referring now to Fig. 11a, there is shown a short segment of an elongate flexible film base lamina or microstructure device 302 which extends lengthwise beyond the range of the drawing, as indicated by broken lines extending from the edges 310, 311 of the short segment. The short segment shown, which is limited by lines 303, includes two successive microchannel arrays 320, 321. Each of the microchannel arrays 320, 321 in this illustration contains four microstructures, two of which are indicated for example at 330, each configured and designed for carrying out a receptor binding assay, as described in detail in Example 1 below, with reference to Fig. 9. Near the edge 310 and associated with each array is a pin registration slot 326, and near the edge 311 and associated with each array is a pin registration hole 327.

Fig. 11b shows a corresponding flexible circuit laminate or microstructure device 304, which also extends beyond the range of the drawing, as indicated by broken lines extending from the edges 312, 313. The short segment shown, which is limited by lines 305, includes two circuit layouts 322, 323, each configured to serve a microchannel array 320, 321 (shown in Fig. 11a) in the assembled device. The flexible circuit laminate can be constructed generally as described above with reference to Figs. 8a, 8b, for example. The circuits consist of conductive traces (two are shown at 332, for example) each connecting a contact terminal (two are shown at 333, for example) to four electrodes (334, for example) each located at a point corresponding to the positions of a reservoir in one of the four microstructures in the array.

Near the edges 312, 313, the flexible circuit laminate 304 is provided with pin registration slots 328 and holes 329, associated with the circuit layouts such that when the base lamina and the flexible circuit laminate are assembled and the respective slots and holes are aligned, precise superposition of the electrodes over the respective reservoirs is ensured. Referring now to Fig. 11c, there is shown generally at 306 a short segment of an embodiment of an assembled continuous form microstructure device of the invention, made by laminating the base lamina of Fig. 11a and the flexible circuit laminate of Fig. 11b. As in Figs. 11a, 11b, the device extends beyond the range of the drawing, as indicted by broken lines extending from edges 314, 315; and the short segment shown, which is limited by lines 307, includes two microstructure arrays 324, 325, each capable of carrying out four receptor binding assays under the control of the associated circuit layout.

The laminate is constructed, as described above, so that the contact terminals are accessible by contact points through contact holes in the cover film. Accordingly, as the laminate is carried through the analytical device, sets of contact points are brought into contact with the corresponding sets of contact terminals on the laminate device. The contact points, in turn, are connected to a source of electrical power, which is provided with controls to change the voltages at the electrodes in a pattern determined according to the sequence of electroflow manipulations to be carried out in the microstructures over the course of the assay.

EXAMPLES

Example 1. Receptor Binding Assay

This Example illustrates a microstructure configuration and method for carrying out a membrane-receptor competitive binding assay according to the invention.

In this Example, cell membrane receptors are attached to solid-phase capture media for facilitating the use of protein receptors in a microfluidic-based assay. Solid-phase attachment of the receptor can be achieved in one of several ways, including, *e.g.*, the use of activated paramagnetic beads or other synthetic particles.

This assay is particularly applicable for receptors belonging to the seven transmembrane family or similar proteins wherein the sequence of amino acids traverses the membrane multiple times. These targets, *e.g.*, the G-protein coupled receptor (or GPCR), are more likely than others to require the physical environment of the membrane lipid bilayer for

physiologically relevant interactions. The dopamine receptor is a specific example of the broader class of GPCR proteins.

A membrane-receptor competitive-binding assay in regard to the above is provided. The non-isotopic assay comprises of two binding events. The primary receptor-ligand affinity reaction can be written generally as:

$$\underbrace{L_i + L^{\bullet}}_{\text{"free" in supernatant}} + \underbrace{(R) = (R)\text{-}L^{\bullet} + (R)\text{-}L_i}_{\text{"bound" in solid-capture phase}}$$

where the library test compound L_i and labeled ligand L• compete for receptor binding sites (R) on the immobilized cell membrane protein. Once the unbound ligand L•, which remains "free" in the supernatant, is removed, then the bound ligand, which is complexed with the immobilized receptor beads, can be detected using a fluorophore-labeled secondary binding protein. If a biotinylated ligand is employed in the primary bioaffinity reaction, then solid-phase fluorescence detection is possible based on the following binding reaction:

$$(R)\text{-}L^{\bullet} + \supset^{*} \rightarrow (R)\text{-}L^{\bullet}\supset^{*}$$

where $\supset^{*}$ represents, for example, an avidin-fluorescein conjugate, as the other member of the secondary specific binding pair. Other protocols based on methods of the invention are also possible. For example, a detection scheme may be employed based upon depletion monitoring of the labeled ligand L•.

Such an assay can be carried out using a microfluidic assay device according to the invention, configured, in one embodiment, as shown generally at 100 in Fig. 9. Referring now to Fig. 9, there is shown an assay laminate or microstructure device 100, on which the microstructure is formed. The microstructure includes chambers and reservoirs that are connected in fluid communication by microchannels. Particularly, device or card 100 includes a zone 125 in which incubation is carried out and separation and detection can be carried out; a secondary capture and detection zone 135; a number of inlet reservoirs: reservoir 102, which serves as a supply of buffer solution; a reservoir 104, serving as a source of library test compound ligand i; reservoir 106, serving as a source of a biotin-labeled ligand conjugate, or biotinyated tracer; reservoir 108, serving as a source of fluorophore-labeled secondary binding protein, or fluorescent tracer; reservoir 110, serving as a source of bead-immobilized, membrane-bound receptor; wash buffer reservoir 112; reservoir 114, serving as a source of an agent that cleaves the fluorophore tag from the fluorescent tracer conjugate; and capture

compound source reservoir 116; and a number of outlet reservoirs: reservoir 124, to receive waste from the binding assay from the fluorescent tracer conjugate; reservoir 126, to receive waste capture compound; and reservoir 128, to receive waste supernatant from binding.

Each reservoir can be provided with an electrode that is connected to a source of electrical power, and potential differences among the various electrodes can be controlled and manipulated to selectively induce electrokinetic transport to and from the reservoirs and within the microchannels and chambers.

In preparation for the assay, the receptors are immobilized as follows. Magnetic latex beads, preactivated to covalently bind protein, are bound to a lectin such as wheat germ agglutinin (WGA). Upon completion of this step, unreacted or exposed bead surface is blocked from nonspecific interactions by incubation with a saturating concentration of carrier protein such as bovine serum albumin or gelatin. Then the WGA coated beads are co-incubated with coil membranes having on them the receptor of interest. This interaction may conclude with an additional blocking step, to remove or inactivate potential sites of nonspecific binding.

With reference again to Fig. 9, the bioanalytical assay proceeds on the microfluidic device 100 as follows.

1. A fixed quantity of receptor-bound beads are introduced into reservoir 110. Then the beads are transferred, by means of an applied magnetic field or electrokinetic flow, to chamber 125 by way of a microchannel in fluid communication with the reservoir and the chamber. In this particular assay protocol, the beads are held in chamber 125 for the duration of the procedure.

2. Next, the compound L_i to be tested for binding ability is moved from reservoir 104 by electrokinetic means through communicating microchannels into chamber 125; and either concurrently therewith or thereafter, a standard compound L^* of known binding properties, is moved from reservoir 106 into chamber 125. This latter compound L^* contains a member of a directly or indirectly detectable signal-producing system, for example, covalently attached biotin.

3. After an appropriate series of electrokinetically driven wash steps using wash buffer moved from reservoir 112, a determination is made for the amount of unknown compound L_i that binds by determining the degree to which it displaces the standard compound L^*. This is measured by introducing the secondary fluoro-labeled binding protein into reaction chamber

125 from reservoir 108 and allowing the complex of compound and receptor, (R)-L•, to react with the streptavidin which binds biotin with high affinity. The amount of streptavidin captured is monitored directly when a fluorescent label is associated with the streptavidin.

4. In some embodiments of the assay in this Example, the amount of fluorescent label associated with the membranes is determined by direct measurement in the capture zone. In other forms of the assay, the fluorescent label may be attached via a disulfide bond (denoted by "~"). This bond is readily cleaved under reducing conditions. Accordingly, dithiothreitol, or beta mercaptoethanol stored in reservoir 114 may be used to release the fluorescent label (denoted by "*").

5. The fluorescent labeled species can then be separated from other reactants by electrokinetic or hydrodynamic enhanced electroseparation techniques. To facilitate detection, the magnetic beads may be immobilized at a site along the capillary path 125 by application of a magnetic field. The fluorescent label may be detected at that site or at a site 135 downstream therefrom. The fluorescent label may be detected in the fluorescent labeled species, or the fluorescent label may be cleaved and detected separately.

Example 2. Enzyme Assay.

This Example illustrates a microstructure configuration and method for carrying out an enzyme assay according to the invention, which can be particularly useful in high-throughput pharmaceutical drug discovery and screening applications.

In this Example, an enzyme, a labeled substrate, and an inhibitor are mixed and allowed to incubate, and then the labeled product of the enzymatic reaction and the labeled unreacted substrate are separated electrophoretically and each is quantitatively determined by detection of the label.

Such an assay can be carried out using a microfluidic assay device according to the invention, configured, in one embodiment, as shown generally at 200 in Fig. 10. Referring now to Fig. 10, there is shown an assay laminate or microstructure device 200 on which the microstructure is formed. The microstructure includes an incubation chamber 250, an injection cross 275, an electrophoretic separation channel 285, and detection zone 295, connected in fluid communication by microchannels with a number of reservoirs, including inlet reservoirs: reservoir 202, for supply of enzyme, which is usually a kinase, and containing ATP and Mg^{2+}; reservoir 204, for supply of labeled substrate S*, which is usually a fluorophore-labeled

peptide; reservoir 206, for supply of enzyme inhibitor; reservoir 218, serving as a supply of assay buffer, and employed to electrokinetically transport the product mixture stream to an outlet reservoir 228; and reservoir 236, serving as a supply of running buffer, and employed to electrokinetically transport a metered plug of the product mixture into the separation channel 285 and the outlet reservoir 246; and a number of outlet reservoirs: reservoir 214, to receive a mixture of excess enzyme, substrate, and inhibitor; reservoir 228, for receiving product mixture stream; and reservoir 246, for receiving detection product waste.

Each reservoir can be provided with an electrode that is connected to a source of electrical power, and potential differences among the various electrodes can be controlled and manipulated to selectively induce electrokinetic transport to and from the reservoirs and within the microchannels and chambers.

In some particularly useful embodiments, the enzyme inhibitor is a pharmaceutical drug candidate, and the assay is carried out to determine the effectiveness of the candidate as an inhibitor for the particular enzyme. Usually the enzyme is a tyrosine specific protein kinase such as, for example, Src kinase; and usually the labeled substrate is a fluorophore-labeled peptide such as, for example, cdc-2 peptide.

The enzyme assay proceeds on the microfluidic device 200 as follows.

1. Mixing. Reagents are moved electrokinetically from inlet reservoirs 202 (enzyme), 204 (substrate), and 206 (inhibitor) toward outlet reservoir 214. Mixing of the reagents occurs in mixing cross 225 and in incubation chamber 250.

2. Incubation. The fluid flow is halted electrokinetically by adjustment of the various potentials in order to let enzyme, substrate and inhibitor incubate in incubation chamber 225.

3. Injection. A continuous stream of the product and excess reagent mixture are moved out from the incubation chamber 250 and into the outlet reservoir 228, using the inlet reservoir 218 as the source of the assay buffer to electrokinetically drive the fluid transport.

4. Separation. A plug of the product mixture is electrokinetically injected from the injection cross 275 into the electrophoretic separation channel 285 and then into waste outlet reservoir 246 using inlet reservoir 236 as the source of the running buffer to electrokinetically drive the fluid transport. As a result of mobility shift produced by conversion of labeled substrate S* to product P*, S* and P* are separated electrophoretically as they are electrokinetically transported in separation channel 285. Laser-induced fluorescence monitoring of the labeled substrate and product is achieved in the detection zone 295. Because

the mobility shift is usually expected to result from differences in charge/mass ratio between S* and P*, a gel matrix is usually not required to achieve separation.

As the Examples illustrate, the invention is useful in a wide variety of applications involving techniques and protocols in fields of, for example, cell biology, molecular biology, HLA tissue typing, and microbiology. More specifically, for example, the invention can be applied to techniques for immunodiagnostics, DNA purification from whole blood and other samples, mRNA isolation, solid phase cDNA synthesis, receptor-binding assays, drug screening and discovery, and cell isolation.

Other embodiments are within the following claims. For example, assay devices other than microchannel devices can be adapted in a continuous form assay array format generally as described herein, to provide high throughput systems. For example, the fluids (reagents, samples, *etc.*) employed in the assay can be mixed and measured in wells (that is, in cavities) constructed in an elongate laminate device, and not necessarily directed by microfluidic manipulation.

And, for example, microstructures or arrays of microstructures may be formed in more than one lamina in the laminated device according to the invention, so that microstructures in one lamina are superimposed over microstructures in another. The superimposed microstructures may, for example, carry out different but related processes or process steps in a fluidic process and, by providing for fluid communication between the laminae, fluids may be transported from one microstructure to another in the course of the process. This permits related processes to be carried out in close proximity under similar conditions, and without a need for transfer of products or byproducts or intermediates from one reaction container (or from one microstructure) to another. Fluid communication between laminae can be provided by, for example, simply perforating the layer that separates the microstructures, and control of the flow through such a perforation can be done, preferably in a valveless fashion, by any of the various means employed for moving fluids within the microstructure of a lamina.

As will be appreciated, although the device according to the invention is described above as being used in continuous processing form, individual microstructures or arrays in an elongate laminate made as described above can be separated one from another, and used as discrete devices in "card" form, each containing a microstructure or an array of microstructures. As may be desired, the elongate laminate may, where such use is contemplated, be made easily separable between successive microstructures or arrays, for

example by perforating or scoring the laminate, or cutting the laminate partway through. Use of the laminate in this way preserves the advantages of continuous form in the manufacture of the device, and replaces advantages of using the device in continuous form with advantages of handling discrete card-form microfluidics devices.

Approaches to aligning the laminae during manufacture other than through holes or notches can be used, for example, techniques employing optical, electrical, and ultrasonic alignment, or employing other mechanical means such as ratchets.

It should be appreciated that any of the microstructure devices described above, including those manufactured in accordance with the processes shown in Figs. 5 and 6 and described above, can be cut or diced into a plurality of discrete card-like microstructure devices, each having a plurality and more specifically an array of discrete microstructures formed therein. Such card-like devices can be used for any of the uses described above. Although such card-like devices can be of any suitable size, in one preferred embodiment such devices can be sized on the order of a credit card.

Another embodiment of the microstructure device of the present invention is shown in Figs. 12 and 13. Microstructure device 406 therein is formed from a laminated structure having a plurality of separate layers or laminae joined together. Microstructure device 406 is preferably a discrete or card-like device, but can also be an elongate flexible device suitable for storage on a reel. The microstructure device 406 is for use with a contact probe assembly 409 having a plurality of contact probes 411 arranged in a predetermined pattern on any suitable support structure 412, shown in Fig. 12 as being a body 412 having a planar surface 413. The elongate contact or electrode probes 411 are made from any suitable conductive metal and in the embodiment of the contact probe assembly 409 shown in Fig. 12 are needle-like in conformation and preferably compliant vertically to facilitate electrical coupling with microstructure device 406. Each of the probes 411 is formed with a rounded end 411a. The probes 411 extend perpendicularly from surface 413 in a predetermined pattern. Although probes 411 are shown as rigidly mounted on support structure 412 so as to remain static during operation, the probes can be mounted on the support structure 412 for retraction and extension from a plurality of bores that open onto surface 414. Separate electrical leads (not shown) are carried by support structure 412 for connection to each of the contact probes 411. Such leads are, in turn, connected serially or separately to a controller (not shown) which

provides appropriate electrical signals, preferably in the form of voltage potentials, to the probes 411.

Microstructure device 406 has a thickness ranging from approximately 100 microns to three millimeters and preferably ranging from approximately 150 microns to one millimeter. The microstructure device 406 includes a laminate structure 421 having first and second spaced-apart planar surfaces 422 and 423 which form two exterior surfaces of the laminate structure 421 (see Fig. 13). A first layer or lamina 426 is included within laminate structure 421. The first lamina or card body 426 is made from any suitable nonconductive material such as plastic and can be relatively rigid or flexible depending on the particular use of microstructure device 406. In one embodiment of a card-like device 406, the first lamina 426 is relatively rigid to provide rigidity to the device 406. Alternatively, other layers in the laminate structure 421 can be relatively rigid, in addition to or instead of a rigid lamina 426, if a rigid microstructure device 406 is desired. First lamina 426 has a first planar surface in the form of first or top surface 422 and a second planar surface 427 spaced apart from the top surface 422 and interior of the laminate structure 421.

The laminate structure is provided with at least one microstructure 428 of capillary dimensions, and preferably a plurality of microstructures 428, formed therein and extending in a direction parallel to the parallel surfaces 422 and 427 of the first lamina 426. For simplicity, only one microstructure 428 is shown in Fig. 12. More specifically, each of the microstructures 428 is formed in first lamina 426 and extends through one of the planar surfaces 422, 427 of the first lamina. As shown in Fig. 13, microstructures 428 open onto second or lower surface 427 of the first lamina 426. Each microstructure 428, as shown in Fig. 12, preferably includes at least first and second microchannels 431 and 432 which meet at an intersection 433. Laminate structure 421 is provided with at least one and as shown a plurality of holes or wells 436 in fluid communication with each microstructure 428. In one preferred embodiment of microstructure device 406, first and second wells 436a and 436b are provided at the first and second end portions of first microchannel 431 and third and fourth wells 436c and 437d are provided at the first and second ends of second microchannel 432. It should be appreciated that the wells 436 can be provided at other locations within microstructure 428 and be within the scope of the present invention. Each of the wells 436, as shown with respect to first well 436a in Fig. 13, is adapted to receive a fluid and consists of a bore extending between surfaces 422, 427 of first lamina 426 and is accessible from the top

surface 422 of the laminate structure 421. Wells 436 can be sized to receive approximately one microliter of such fluid.

Laminate structure 421 includes a second layer or lamina 441 made from any suitable non-conductive material such as plastic. Second lamina or film 441 has a first planar surface 442 and a second planar surface in the form of second or bottom surface 423 which is spaced-apart from and parallel to the top surface 422. Second lamina 441 is secured to first lamina 426 by any suitable means such as an adhesive layer 443 disposed between surfaces 427 and 442. In an alternative embodiment, surfaces 427 and 442 can be diffusion bonded together and adhesive layer 443 thus eliminated.

A plurality of electrical means 444 are at least partially carried by second lamina 441. The electrical means 444 are preferably equal in number to the number of wells 436 provided in laminate structure 421 such that each of the wells 436 has a corresponding electrical means 444. Each of such electrical means 444 includes an electrode portion 444a in communication with any fluid provided in the well 436 and a contact or pad portion 444b spaced apart from electrode portion 444a and not in contact with any such fluid in well 436. An interconnect portion 444c connects the electrode portion 444a to the contact portion 444b. In the embodiment of the microstructure device shown in Figs. 12 and 13, each electrical means 444 extends through a bore 446 between surfaces 442 and 423 of the second lamina 441 such that the electrical means resembles a circular plug or disk disposed in the second lamina 441. Bore 446 has a diameter smaller than the diameter of well 436 so as to minimize fluid contact with the material of electrode portion 444a. The electrical means 444 are each made from any suitable material such as conductive carbon ink. Conductive metals such as silver, copper, gold, platinum and palladium, other conductive inks such as metalized inks and blends of conductive materials and polymers such as conductive epoxies and conductive adhesives are also suitable materials for electrical means 444. Electrode portion 444a is disposed adjacent first or upper surface 442 of the second lamina and interconnect portion 444c is disposed in bore 446. Contact portion 444b is disposed adjacent bottom surface 423 of the second lamina 441 and underlying the electrode portion. The contact portion 444b can extend downwardly from bottom surface 423 and have a rounded end as shown in FIG. 13. The diameter of the contact portion 444b is larger than bore 446 so that a portion of the contact portion sits on the bottom surface 423 for facilitating retention of the electrical means 444 in bore 446 during engagement with contact probes 411. Electrode portion 444a and contact portion 444b are

aligned with the respective well 436 and electrode portion 444a forms at least a portion of the bottom surface of such well 436.

Contact portions 444b are accessible from the exterior or bottom surface 423 of laminate structure 421 and microstructure device 406 without need of penetrating any of the layers of such structure 421 and device 406. In addition, contact portions 444b are arranged on bottom surface 423 in a pattern which corresponds to the predetermined pattern of contact probes 411. As such, the contact probes 411 can register with and simultaneously or otherwise engage respective contact portions 444b when microstructure device 406 and support structure 412 are moved relative to each other into close proximity with each other.

Microstructure device 406 can optionally include a third layer or lamina 448 made from any suitable material such as plastic. The third lamina 448 overlies each of wells 436 and is secured to laminate structure 421 by any suitable means such as heat bonding so as to suitably secure any fluid located within the wells. Alternatively, the cover lamina 448 can be removably or temporarily secured to the laminate structure 421 by an adhesive or any other suitable means to permit removal and reattachment of the cover lamina. The third lamina 448 has a first or upper planar surface 451 which serves as an exterior surface of microstructure device 406 and a second or lower planar surface 452 which is adhered to top surface 422 of laminate structure 421 by a pressure sensitive adhesive, heat bonding or any other suitable means.

In operation and use, a fluid and preferably a liquid is provided in each well 436. A fluid 453 is shown in Fig. 13 in first well 436a. Such fluids can be supplied to wells 436 during manufacture of microstructure device 406 or immediately prior to use of the device 406 and can be a single fluid or a plurality of fluids of different composition. Fluids can be sealed in the wells 436 by means of third or cover lamina 448. Cover lamina 448 permits fluids to be supplied to wells 436 during manufacture of the device 406 and stored therein during transport. Prior to use, the cover lamina 448 can be pierced if additional fluids need be added to one or more wells 436 or, if the cover lamina 448 is removable, removed for the supply of such additional fluids and optionally reattached thereafter. Cover lamina 448 advantageously inhibits evaporation of fluids contained in wells 436 and microstructures 428.

Contact portions 444b and contact probes 411 are brought into engagement to permit electrical coupling thereof. In this regard, microstructure 406 can be placed upon contact probes 411 or, alternatively, contact probes 411 brought into contact with the microstructure

device 406. In either instance, contact probes 411 simultaneously engage respective contact portions 444b. A force can optionally be applied to the top surface of microstructure device 406 to enhance electrical contact between contact portions 444b and contact probes 411. A distributed force can be applied to the top surface of device 406 by means of pressurizing the top surface in a conventional manner with any suitable fluid such as air or argon gas.

Microstructure devices 406 can be used in any of the processes described or referenced above. During such processes, the fluids provided in wells 436 can be electrokinetically transported through microstructure 428 by means of voltage differentials provided between appropriate wells 436. Probes 411 provide a predetermined voltage potential to one or more electrode portions 444a when such voltage potential is supplied by the controller. The sequence and timing of such voltage potentials determine the manner in which fluids flow through microstructures 428.

It should be appreciated that all or portions of cover lamina 448 and laminate structure 421 can be made from materials which are optically transparent so as to permit optical detection of the fluids within microstructures 428 and/or wells 436. Alternatively, microstructure device 406 can be adapted for use with other conventional detection devices for determining characteristics of the fluids within microstructures 428 and/or wells 436.

Microstructure device 406 permits electrical potentials to be provided to each of the wells 436 therein without the need of contact probes 411 being inserted directly into the fluid within such wells. Instead, electrical probes 411 each engage a contact portion 444b which transmits the electrical potential of the contact probe 411 to electrode portion 444a in contact with the fluid within the well 436. Contact probes 411 are thus not contaminated with the fluid of the wells 436 and can be used in the operation of a second microstructure 406 without fear of mixing the fluids from the first microstructure device with the fluids in the second microstructure device. As can be seen, contact probes 411 can be repeatedly used in a process which sequentially analyzes and/or detects characteristics of fluids supplied to a plurality of microstructure devices 406. The close proximity of the electrode portions 444a to the contact portions 444b inhibit current losses in the electrical means 444.

Microstructure device 406 can be used in the manner discussed above with other contact probe assemblies. For example, a portion of another contact probe assembly 456 is shown in Fig. 13. The assembly 456 is substantially similar to probe assembly 409 except that a plurality of traces pads 457 are arranged on body 413 in a predetermined pattern instead of

contact probes 411. Each pad 457, one of which is shown in Fig. 13, is formed on an electrical trace 458 disposed on surface 414 of the body. The traces 458 are made from any suitable material such as copper, silver, platinum, palladium, conductive carbon or platinum-laden polymers and other conductive inks such as metalized inks and blends of conductive materials and polymers such as conductive expoxies and conductive adhesives formed on surface 414. These materials can be so disposed on surface 414 by vapor deposition, screen or other printing, other traditional flex circuit methods or any other suitable means. Trace pads 457 can be made from any suitable material such as gold and/or the materials listed above for traces 458 and be formed on the end of the respective trace by any of the methods discussed above with respect to the traces 458. The bulbous contact portions 444b of microstructure device 406 depend from bottom surface 423 so as to facilitate electrical contact between the contact portions 444b and pads 457.

In another embodiment, a microstructure device 461 substantially similar to the microstructure device 406 and for use with contact probe assembly 409 is shown in Fig. 14. Like reference numerals have been used to describe like components of microstructure devices 406 and 461. A laminate structure 462 substantially similar to laminate structure 421 is included within microstructure device 461. A second layer or lamina 463 is included in laminate structure 462 and has a first or upper planar surface 466 and a second planar surface in the form of bottom surface 423 of the laminate structure 462. Although the second lamina 463 is shown as being secured to first lamina 426 by an adhesive layer 467, it should be appreciated that surfaces 427 and 466 can be heat bonded or sealed together by any other suitable means. Microstructure device 461 is shown with a cover lamina 448, but it should be appreciated that the device 461 can be provided without a cover lamina 448 so that wells 436 are each accessible from the top surface or exterior of microstructure device 461.

A plurality of electrical means 471 are at least partially carried by second lamina 463. The electrical means 471 are preferably equal in number to the number of wells 436 provided in laminate structure 462 such that each of the wells 436 has a corresponding electrical means 471. Each of such electrical means 471 includes an electrode portion 471a which can communicate with the fluid supplied to the well 436 and a contact or pad portion 471b spaced-apart from electrode portion 471a and not in fluidic contact with any such fluid. Electrical means 471 each include a trace portion or trace 471c which electrically connects the respective electrode 471a to the contact portions 471b. As can be seen from Fig. 14, contact portion 471

is disposed adjacent and more specifically formed on bottom surface 423. Electrical trace 471c extends from the contact portion through a passage 472 extending transversely and more specifically diagonally between surfaces 466 and 423 of the second lamina 463 and has a further portion disposed on the upper surface 466 underlying electrode portion 471a. Second lamina 463 can be made from any suitable flex circuitry material such as acrylic, polyimide or PET. Contact portions 471b and portions of trace 471c can be formed from any suitable material such as copper, silver, platinum, palladium, conductive carbon or platinum-laden polymers and other conductive inks such as metalized inks and blends of conductive materials and polymers such as conductive expoxies and conductive adhesives formed on the aforementioned surfaces of second lamina 463. These materials can be so disposed on such surfaces of the second lamina by vapor deposition, screen or other printing, other traditional flex circuit methods or any other suitable means. Electrode portions 471a can be formed from any suitable material such as gold and/or the materials listed above for contact portions 471b and traces 471c and be formed on trace 471c by any suitable means such as those described above with respect to contact portions 471b and traces 471c. Each of the electrode portions 471a is shown as forming at least a portion of the bottom surface of the respective well 436. Alternatively, the electrode portions 471a can form the entire bottom surface of the well 436 or merely make fluidic contact with the well from a side wall or otherwise. Contact portions 471b are accessible from the exterior or bottom surface 423 of laminate structure 461 and microstructure device 461 and are each preferably spaced-apart from the centerline of the respective well 436. The contact portions 471b are arranged on the underside of microstructure device 461 in a pattern corresponding to the pattern of contact probes 411 on support structure 412.

Microstructure device 461 can be operated with contact probes 411 in substantially the same manner as described above with respect to microstructure device 406. Rounded ends 411a of the contact probes 411 can simultaneously engage the contact portions 471b for providing the desired electrical potential to the fluid in each of wells 436.

Microstructure device 461 can also be used in the manner discussed above with other contact probe assemblies. For example, a portion of another contact probe assembly 476 is shown in Fig. 14. The assembly 476 is substantially similar to probe assembly 409 except that a plurality of traces pads 477 are arranged on body 413 in a predetermined pattern instead of contact probes 411. Each pad 477, one of which is shown in Fig. 14, is formed on an

electrical trace 478 disposed on surface 414 of the body. The traces 478 are made from any suitable material such as those described above with respect to traces 457 of contact probe assembly 456 and are disposed on surface 414 by any suitable method such as those discussed above with respect to traces 457. Trace pads 477 can be made from any suitable material such as those described above with respect to electrical means 444. The bulbous trace pads 477 extend upwardly from surface 414 and traces 478 of contact probe assembly 476 so as to facilitate electrical contact between the contact portions 471b and trace pads 477.

A further embodiment of a microstructure device of the present invention is shown in Figs. 15 and 16. Microstructure device 481 therein is substantially similar to microstructure devices 406 and 461 and is for use with contact probe assembly 409. Like reference numerals have been used to describe like components of devices 406, 461 and 481. A laminate structure 482 substantially similar to laminate structure 421 is provided in microstructure device 481. Laminate structure 482 includes a first layer or lamina 483 which is substantially similar to first lamina 426 and has first and second planar surfaces 486 and 487 extending in parallel directions. At least one and preferably a plurality of microstructures 428 are provided in laminate structure 482. One of microstructures 428 is shown in Fig. 15 and a portion of such microstructure 428 is shown in Fig. 16. The microstructures 428 are formed in laminate structure 482 in the same manner as they are formed in laminate structure 421. Specifically, each of the microstructures 428 is formed in first lamina 483 and opens onto second or lower surface 487 of the lamina 483. A plurality of wells 436 extend between surfaces 486 and 487 in fluid communication with the microstructure 428 and are each accessible from first or top surface 486 of laminate structure 482.

Laminate structure 482 includes an optional second layer or lamina 488 made from plastic or any other suitable material. Thin film or lamina 488 has a first or upper planar surface 491 and a second or lower planar surface 492 parallel to the upper surface 491. The upper surface 491 is secured to the lower surface 487 of first lamina by diffusion bonding or any other suitable method. A bore 493 having a diameter substantially equal to the diameter of the well-forming bore in first lamina 483 extends between surfaces 491 and 492 for forming a part of well 436b. The combined thicknesses of laminae 483 and 488 determine the depth of wells 436. If a thin layer film is used for second lamina 488, the thickness of first lamina 483 can be increased to provide the desired depth to wells 436.

A third layer or lamina 496 is included in laminate structure 482. The third lamina 496 is substantially similar to second lamina 463 and has a first or upper planar surface 497 and a second or lower planar surface 498. Upper surface 497 of the third lamina 496 is secured to lower surface 492 of second lamina 488 by an adhesive layer 499 or any other suitable means. Upper surface 486 of the first lamina and lower surface 498 of the third lamina 496 form the top and bottom surfaces of laminate structure 482.

A plurality of electrical means 501 are at least partially carried by third lamina 496 for each microstructure 428 such that each of the wells 436 has a corresponding electrical means 501. More specifically, each electrical means 501 is disposed on upper surface 497 of the third lamina 496. Each such electrical means 501 has an electrode portion 501a, a pad or contact portion 501b and a trace portion or trace 501c. The electrical trace 501c is made from any suitable material such as any of the materials discussed above with respect to contact portions 471b and traces 471c and is disposed on upper surface 497 by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c. The trace 501c has a first end portion underlying the respective well 436 and a second spaced-apart end portion underlying an access bore 502 extending between upper and lower surfaces 486 and 487 of the first lamina 483 and an access bore 503 extending between upper and lower surfaces 491 and 492 of second lamina 488. Electrode portion 501a consists of a layer of material disposed on the first end portion of trace 501c underlying well 436. Electrode portion 501a is shown in Fig. 16 as forming at least a portion of the lower surface of the well 436. Contact portion 501b consists of a layer of material disposed on the opposite second end portion of trace 501c and serves as the lower surface of access bores 502 and 503. Adhesive layer 499 extends around the base of bore 493 and over the portion of trace 501c between electrode portion 501a and contact portion 501c to provide a fluid seal at the bottom of the well 436. The electrode portion 501a and the contract portion 501b can each be made from any suitable material such as any of the materials discussed above with respect to electrode portions 471a and can be formed by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c. Contact portions 501b are arranged on the bottom surface 498 of laminate structure 482 in a pattern corresponding to the pattern of contact probes 411 on support structure 412 and are accessible from such bottom surface 498.

Microstructure device 481 can optionally include a fourth layer or lamina 506 substantially similar to cover lamina 448 and having a first or upper surface 507 and a second

or lower surface 508 heat bonded or otherwise suitably secured to upper surface 486 of the first lamina 483. Cover lamina 506 overlies each of wells 436 so as to sealably secure the fluid 453 within the well. An opening 509 is provided in cover lamina 406 in registration with access bores 502 and 503 for permitting contact probes 411 to engage contact portions 501b.

Microstructure device 481 can be operated in substantially the same manner as described above except that contact probes are disposed above the device 481. In this regard, contact probes 411 are positioned above microstructure device 481 such that rounded ends 411a of the contact probes 411 face downwardly toward openings 509 and contact portions 501b. When it is desired to transport fluids within microstructures 428, the microstructure device 481 and contact probes 411 are moved relative to each other such that rounded ends 411a enter openings 509 and electrically engage contact portions 501b.

The engagement of contact probes 411 with the top of microstructure device 481 allow less obscured access to the bottom of device 481 for purpose of optical detection and/or temperature control. Second lamina 488 provides an opposing surface 491 to the microstructures 428 formed in the first lamina which is not an adhesive. The inclusion of the second lamina 488 facilitates forming microstructures 428 from walls that are all of the same material, which can be advantageous in certain processes of device 481. In addition, the absence of fluid contact with the adhesive permits a broader selection of adhesives to be considered for adhesive layer 499.

An embodiment of another microstructure device is shown in Fig. 17 where a portion of microstructure device 521 is depicted. The microstructure device 521 is substantially similar to microstructure device 406 and like reference numerals have been used to describe like components of devices 406 and 521. A laminate structure 522 substantially similar to laminate structure 421 is provided. A first lamina 426 having a plurality of microstructures 428 formed therein is included in the laminate structure 522. For simplicity, a portion of only a single microstructure 428 and one of the plurality of wells 436, specifically first well 436a, is shown in Fig. 17. A second layer or lamina 523 made from any suitable flex circuit material such as acrylic, polyimide or PET is included within laminate structure 522 and has a first or upper planar surface 526 and a second or lower planar surface in the form of bottom surface 423 of the laminate structure 522. Although the second lamina 523 is shown as being secured to first lamina 426 by an adhesive layer 527, it should be appreciated that surfaces 427 and 528 can be heat bonded or sealed together by any other suitable means. Microstructure device 521 is

shown with a cover lamina 448, but it should be appreciated that the device 521 can be provided without a cover lamina so that wells 436 are each accessible from the top surface or exterior of microstructure device 521.

A plurality of electrical means 531 for each microstructure 428 are at least partially carried by second lamina 523 such that each of the wells 436 has a corresponding electrical means 531. More specifically, each electrical means 531 is disposed on upper surface 526 of the second lamina 523. Each such electrical means 531 has an electrode portion 531a, a pad of contact portion 531b and a trace portion or trace 531c. Trace 531c is made from any suitable material such as any of the materials discussed above with respect to contact portions 471b and traces 471c and is disposed on upper surface 526 by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c. The trace 531c has a first end portion underlying the respective well 436 and a spaced-apart second end portion underlying a recess or cavity 532 formed in first lamina 426 and opening onto lower surface 427 thereof. Electrode portion 531a consists of a layer of material deposited on the first end portion of trace 531c underlying well 436 and is shown in Fig. 17 as forming at least a portion of the lower surface of well 436. Contact portion 531b consists of a layer of material disposed on the second end portion of trace 531c and preferably extends across the entire opening of recess 532 in lower surface 427. The electrode portion 531a and the contract portion 531b can each be made from any suitable material such as any of the materials discussed above with respect to electrode portions 471a and can be formed by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c.

Microstructure device 521 is for use with a contact probe assembly (not shown) having piercing contact probes 537 and otherwise substantially similar to contact probe assembly 409. Contact probes 537 are substantially similar to contact probes 411 except that the probes 537 are capable of piercing the second lamina 523 and electrical means 531. Piercing contact probes 537 can have sharpened tips 537a. A portion of one contact probe 537 is shown in dashed lines in Fig. 17. Like contact probes 411, the probes 537 are arranged on support structure 412 in a predetermined pattern.

The second lamina 523 has a thickness and hardness which permits sharpened tips 537a of the contact probes 537 to penetrate the second lamina 523. Contact portions 531b and the portion of traces 531c thereunder are also of a thickness which permits penetration by

sharpened tips 537a. Contact portions 531b are arranged on microstructure device 521 in a pattern corresponding to the pattern of contact probes 537. In a preferred embodiment, the number of contact probes 537 is at least equal to the number of contact portions 531b.

Microstructure device 521 can be operated and used in a manner described above. When it is desired to electrokinetically transport the fluids within wells 436 of the microstructures 428 in the device 521, the operator causes relative movement between the structure device 521 and the contact probe assembly so that sharpened tips 537a of the contact probes 537 penetrate second lamina 523 and contact portions 531b and thus make electrical contact with electrical means 531. Thereafter, desired voltage potentials can be applied to the fluids in wells 436. The placement of puncturable contact portion 531b internally of microstructure device 521 eliminates exposed contact portions, which can be damaged from handling. The puncturable lamina 523 eliminates the need of access bores through other layers of laminate structure 522, which can add cost to the device 521.

In another embodiment of the invention, microstructure device 551 for use with contact probe assembly 409 is shown in Figs. 18 and 19. Microstructure and device 551 is preferably a card-like device, but can also be an elongate flexible device suitable for storage on a reel. As such, microstructure device 551 can have a size and shape similar to microstructure device 406. The device 551 includes a laminate structure 552 having a first or top planar surface 553 and a second or bottom planar surface 554 spaced apart from the top planar surface 553. The surfaces 553 and 554 form a portion of the exterior of the laminate structure. A first layer or lamina 557 is included within laminate structure 552 and is made from any suitable non-conductive materials such as plastic. The first lamina 557 can be relatively rigid or flexible depending on the particular use and configuration of the microstructure device 551. Alternatively, other layers in laminate structure 552 can be relatively rigid, in addition to or instead of a rigid lamina 557, if a rigid microstructure device 551 is desired. First lamina 557 has a first planar surface in the form of first or upper surface 558 and a second planar surface 554 spaced-apart from the upper surface 558.

The laminate structure 552 is provided with at least one microstructure 428 and preferably a plurality of microstructures 428 formed therein and extending in a direction parallel to the parallel surfaces 558 and 554 of the first lamina 557. A plurality of three microstructures 428, namely first microstructure 428a, second microstructure 428b and third microstructure 428c, are shown in Fig. 18. A portion of third microstructure 428c is shown in

Fig. 19. Each of the microstructures 428 is formed in first lamina 557 and opens through one of the planar surfaces 554, 558 of the first lamina. In the embodiment shown, the microstructures 428 open onto upper surface 558 of the first lamina 557. Laminate structure 552 has at least one and as shown a plurality of holes or wells 561 substantially similar to wells 436. Specifically, first and second wells 561a and 561b are provided at the first and second ends of first microchannel 431 and third and fourth wells 561c and 561d are provided at the first and second ends of second microchannel 432. It should be appreciated that wells 561 can be provided at other locations within microstructure 428. For example, a fifth well 561e is provided in an intermediate portion of first microchannel 431 between wells 561a and 561b.

Laminate structure 552 has a second layer or lamina 566 made from any suitable non-conductive material such as plastic overlying first lamina 551. More specifically, second lamina 556 can be made from any suitable flex circuit material such as acrylic, polyimide or PET. The second lamina 556 has a first or upper planar surface 567 and a second or lower planar surface 568 which is spaced-apart from and parallel to upper surface 567. A portion of microstructure device 551 is cut away in Fig. 18 to expose a portion of upper surface 567. Second lamina 566 is secured to first lamina 557 by any suitable means such as heat bonding together surfaces 558 and 568. A plurality of bores 569 extend through surfaces 567 and 568 for forming the first or lower segment of respective wells 561.

A plurality of electrical means similar to the electrical means described above are at least partially carried by second lamina 566. More specifically, such electrical means are carried by upper surface 567 of the second lamina 566 and thus extend in a single plane. A plurality of four electrical means 576-579 are shown in Figs. 18-19. First electrical means 576 includes an electrode portion 576a, a pad or contact portion 576b and a trace portion or trace 576c. Electrical trace 576c is made from any suitable material such as any of the materials discussed above for contact portions 471b and traces 471c disposed on surface 567 by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c. The trace 576c has a plurality of first end portions adjacent the respective first wells 561a of first microstructure 428a, second microstructure 428b and third microstructure 428c. The first end portion of trace 576c in the vicinity of first well 561a for third microstructure 428c is shown in cross-section in Fig. 19. Such trace end portion is annular in shape, although any suitable shape can be provided. An electrode portion 576a of any suitable shape is disposed on the first end portion of each trace 576c. The electrode portion 576a for

first well 561a of third microstructure 428c is annular in shape and extends around the respective bore 569 in the second lamina 566. More specifically, such annular electrode portion 576a is concentrically disposed about the well 561a. An opening is provided in the center of each annular electrode portion 576a for forming part of the respective well 561a. A contact portion 576b is disposed on the second end portion of each trace 576c. The electrode portion 576a and the contract portion 576b can each be made from any suitable material such as any of the materials discussed above with respect to electrode portions 471a and can be formed by any suitable means such as any of those described above with respect to contact portions 471b and traces 471c.

Second electrical means 577 has an electrode portion 577a, a pad or contact portion 577b and a trace portion or trace 577c substantially similar in construction and material to the corresponding portions of first electrical means 576. The electrical trace 577c has a plurality of first end portions adjacent each of second wells 561b and a second end portion in the vicinity of the contact portion 576b of the first electrical means 576. An electrode portion 577a of any suitable shape is disposed on the first end portion of each trace 577c. As can be seen from Fig. 18, the electrode portion 577a for first microstructure 428a is arcuate or horseshoe in shape. Specifically, electrode portions 577a and the portion of traces 577c thereunder each subtend an angle of approximately 90° about the centerline of the respective well 561b. Contact portion 577b is disposed on the second end portion of trace 577c adjacent contact portion 576b.

Third electrical means 578 has an electrode portion 578a, a pad or contact portion 578b and a trace portion or trace 578c substantially similar to the corresponding portions of second electrical means 577. Electrical trace 578c has a first end portion adjacent each of third wells 561c and a second end portion adjacent contact portions 576b and 577b. An electrode portion 578a is deposited on the first end of each trace 578c adjacent the respective well 561c and engages only a portion of the well 561c. Each electrode portion 578a and the portion of the trace 578c thereunder subtend an angle of less than approximately 30° with respect to the centerline of the respective well 561c and are disposed in the well diametrically opposite the entrance of microchannel 432 in the well. Contact portion 578b is deposited on the second end portion of trace 578c in the vicinity of contact portions 576b and 577b. Fourth electrical means 579 has an electrode portion 579a, a pad or contact portion 579b and a trace portion or trace 579c, each formed of the materials of the corresponding portions of the first electrical

means 576 and deposited onto upper surface 567 in the same manner as first electrical means 576. The electrical trace 579c has a first end portion adjacent each of fourth wells 561d and a second end portion in the vicinity of contact portions 576b, 577 and 578b. An electrode portion 579a is deposited on each first end portion of trace 579c adjacent the respective well 561d and, as shown in Fig. 18, has a shape similar to that of electrode portion 578a.

A third layer or lamina 586 is included within laminate structure 552 and overlies second lamina 566. The third lamina 586 is similar in construction, size and composition to second lamina 566 and has a first or upper planar surface 587 and a second or lower planar surface 588 extending parallel to upper surface 587. A portion of microstructure device 551 is cut away in Fig. 18 to expose a portion of upper surface 587. Lower surface 588 of the third lamina 586 is secured to upper surface 567 of the second lamina 566 by an adhesion layer 589, although laminae 566 and 586 can be secured together by any other suitable means such as heat bonding. A plurality of bores 591 extend between upper and lower surfaces 587 and 588 forming the second or intermediate segments of each of the wells 561 of microstructure device 551. Bores 591 each have an inner diameter greater than the inner diameter of bores 569 in the second lamina 566 so that the intermediate segment of wells 561 is larger in diameter than the lower segment of the wells formed by bores 569. The inner diameter of bores 591 is sufficiently large so that electrode portions 576a, 577a, 578 and 579a formed on the second lamina 566 are exposed to the fluid 453 within the wells 561. An opening, shown but not identified in Fig. 18, is provided through surfaces 587 and 588 for permitting access to contact portions 576b, 577b, 578b and 579b through the third lamina 586.

A plurality of electrical means substantially similar to the electrical means on second lamina 566 are at least partially carried by third lamina 586. Specifically, a plurality of fifth electrical means 596 and a plurality of sixth electrical means 597 are carried on upper surface 587 for each of the microstructures 428 formed by laminate structure 552. For simplicity, fifth and sixth electrical means 596 and 597 are shown only with respect to second microstructure 428b and third microstructure 428c in Figs. 18 and 19. The fifth and sixth electrical means 596 and 597 are substantially similar in construction and materials to electrical means 576-579 described above. Each of the fifth electrical means 596 has an electrode portion 596a, a pad or contact portion 596b and a trace portion or 596c. Each electrical trace 596c has a first end portion adjacent the respective first well 561a and a second end portion spaced-apart from the respective well 561a. The first end portion of each trace 596c is annular in shape, although

any suitable shape can be provided, and extends around the first well 561a. An electrode portion 596a which is shown as being annular in shape is deposited on top of the first end portion of each trace 596c. The first end portion of each trace 596c and each electrode portion 596a has an opening in the center thereof forming a part of the respective first well 561a. A contact portion 596b is deposited atop the second end portion of each trace 596c. Each sixth electrical means 597 has an electrode portion 597a, a pad or contact portion 597b and a trace portion or trace 597c. Each electrical trace 597c has a first end portion adjacent the respective fifth well 561e and a second end portion spaced-apart from the well 561e. An electrode portion 597a is disposed atop the first end portion of each trace 597c and is adapted to contact the fluid within the fifth well 561e. In this regard, each electrode portion 597a is substantially similar to electrode portions 577a and 578a described above. A contact portion 597b is deposited atop the second end portion of each electrical trace 597c.

Laminate structure 552 has a fourth layer or lamina 601 made from any suitable material such as plastic which overlies third lamina 586. Lamina 601 can be relatively rigid if a rigid microstructure device 551 is desired. Fourth lamina 601 has a first or upper planar surface consisting of top surface 553 of the laminate structure 552 and a second or lower planar surface 603 extending parallel to the upper surface 553. Lower surface 603 is adhered to upper surface 587 of third lamina 586 by an adhesion layer 604 or any other suitable means. A plurality of bores 607 extend between surfaces 553 and 603 for forming a third or upper segment of each of the wells 561 in microstructure device 551. The bores 607 each have an inner diameter greater than the inner diameter of bores 591 so that the upper segment of the wells 561 is larger in diameter than the lower and intermediate segments of the wells. The inner diameter of bores 607 is sufficiently large such that electrode portions 596a and 597a are exposed so as to contact the fluid within the wells. An additional opening, shown but not identified in Fig. 18, is provided between surfaces 553 and 603 to permit access to contact portions 576b, 577b, 578b and 579b through the fourth lamina 601. A further plurality of bores 609 extend between surfaces 553 and 603 in registration with contact portions 596b and 597b to permit access to the fifth and sixth electrical means 596 and 597.

The aggregate thicknesses of laminae 566, 586 and 601 determine the depth of wells 561. Second and third laminae 566 and 586 can each have a thickness ranging from 40 to 250 microns. Fourth lamina 601 can have a thickness ranging from 250 microns to one millimeter. As can be seen, laminae 566 and 586 can be films backing a thick fourth lamina 601.

A fifth layer or lamina 611 is included in microstructure 551 for serving as a cover layer. Cover lamina 611 is substantially similar to cover lamina 448 described above and has a first or upper planar surface 612 and a second or lower planar surface 613 extending in a direction parallel to upper surface 612. Lower surface 613 is secured to upper surface 553 of the fourth lamina 601 by heat bonding or any other suitable means. An opening 616 extends through surfaces 612 and 613 to permit access to contact portions 576b, 577b, 578b and 579b. In addition, a plurality of bores 617 extend between surfaces 612 and 613 in registration with bores 609 to permit access to contact portions 596b and 597b. The contact portions of electrical means 576-579 and 596-597 and wells 561 are accessible from top surface 553 of the laminate structure 552. It should be appreciated that microstructure device 5551 can be provided without a cover lamina 611 and be within the scope of the present invention.

One or more optional detection bores 621 can extend through any or all of cover lamina 611, third and fourth laminae 586 and 601 and adhesive layers 589 and 604 for each microstructure 428 to facilitate optical detection by a detector (not shown) of fluid within microstructures 428. One such bore 621 is shown in dashed lines in Fig. 19. Such bores minimize undesirable fluorescence which can be provided by such layers and adhesives.

Although microstructure device 551 is shown and described as having first and second laminae or flex circuit layers 566 and 586, electrical means 576-579 can be formed on upper surface 558 of first lamina 557 by any suitable manner, such as any of the methods described above, so as to eliminate second lamina 566. Alternatively, electrical means 576-579 can be formed on lower surface 588 of third lamina 496, the invention being broad enough to cover overlapping electrical means of the type described above separated by an insulating or nonconductive layer.

In operation and use, microstructure device 551 can be used with electrode assembly 409 for any of the processes and methods described above. In this regard, rounded ends 411a of the contact probes 411 are extended through top surface 553 of the laminate structure to simultaneously engage contact portions 576b, 577b, 578b, 579b, 596b and 597b. Appropriate voltage potentials are then applied to the fluids 453 within wells 561 to electrokinetically move fluids with the plurality of microstructures 423 provided in microstructure device 551.

During such operation, each of traces 576c, 577c, 578c and 579c permit a single contact probe 411 to be utilized for providing a voltage potential to the respective plurality of wells 561 electrically coupled thereto. Fifth well 561e and sixth electrical means 577 can be

utilized to assist the movement of fluid within microstructure 551 between the first and second end portions of first microchannel 431. The location of the electrode portions in the well at a point farthest from the opening of the microstructure 428 in the well, such as electrode portions 578a which is diametrically opposite the opening of the respective microstructures, enhances electrokinetic movement of fluids into and from the well by maximizing the amount of fluid in the relevant microchannel which is between the operative electrode portions. Arcuate or horseshoe-shaped electrode portions, such as electrode portions 577a, can be similarly disposed opposite the microstructure opening in the well to focus the electrical potential towards the microchannel of the microstructure.

The wells 561 in microstructure device 551 are formed in layers other than the layer(s) forming microstructures 428. It has been found that such wells 561 can be more easily manufactured, for example in a punching operation, when not present in the layer forming microstructure or microstructures 428. The depth of wells 561 so formed is determined by the thickness and number of such other layers in laminate structure 552.

The inclusion of two flex circuit layers in laminate structure 552, that is second and third lamina 566 and 586, permit complex and/or dense patterns of electrodes and electrical traces to be provided in microstructure device 551. For example, traces on one of such flex circuits can extend over or under traces on the other such flex circuit, the traces being electrically insulated from each other by one of the lamina of the laminate structure 552. The insulating separation layer minimizes cross talk between the crossing traces. The electrodes, electrical traces and contact pads can also cross over or under the microchannels or other portions of microstructures 428. Such multi-layered electrical patterns permit a greater number of microstructures 428 and/or more elaborate microstructure designs to be provided on a given surface area of microstructure device 551. The first and second flex circuits also permit more than one contact probe to supply a voltage potential to a particular well 561 or other portion or the microstructures 428. For example, a voltage potential can be applied to the fluid 453 in first well 561a of microstructure device 551 by either first electrical means 576 or fifth electrical means 596. The multiple layers of flex circuits can also facilitate placement of the contact portions along one side of the device, such as shown in Fig. 18 with contact portions 576b, 577b, 578b and 579b.

It should be appreciated that the illustrated configurations of electrodes and electrical traces on second and third lamina 566 and 586 can be combined in a multitude of ways to

provide a variety of microstructure devices 551. In this regard, the electrode portions can be sized as desired and can be provided in wells or other portions of microstructures 428. One or more electrode portions can be provided for each well so as to permit one or more voltage potentials to be alternatively or otherwise applied to the well. A single trace can be used to transmit a voltage potential to a single well or to a plurality of wells. More than two flex circuit layers can also be provided in other embodiments of the microstructure device of the present invention.

A microstructure device substantially similar to device 551 can be provided without electrical means of type described above integrated therein. For example, flex circuit layers 566 and 586 can be eliminated from device 551 to provide a laminate with microstructures 428 and wells 561, but not electrical means 576-579 and 596-597.

The invention herein can be broadly claimed as a microstructure device comprising a laminate structure having a first lamina being provided with at least one microstructure extending in a direction parallel to the first and second parallel surfaces of the first lamina. The laminate structure is provided with a plurality of spaced-apart bores in the first lamina or a second lamina for forming at least a portion of a plurality of wells in fluid communication with the at least one microstructure. Electrical means of the type described above is carried by the laminate structure for each of the plurality of wells. Optionally, the first lamina is provided with an additional such microstructure and the laminate structure is provided with an additional plurality spaced-apart bores in one of its lamina for forming at least a portion of an additional plurality of wells in fluid communication with the additional microstructure. Optional additional electrical means can be carried by the laminate structure for each of the additional plurality of wells, the additional electrical means overlying the first-named electrical means and being electrically insulated from the first-named electrical means. An insulating layer of the lamina structure can optionally be disposed between the first-named and additional electrical means.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A continuous form microstructure array device comprising an elongate film laminate having a width bounded by first and second edges and a length bounded by first and second ends, said elongate film laminate having a plurality of microstructures arranged therein, each said microstructure configured to carry out at least one step in a microfluidic process.

2. The continuous form microstructure array device of claim 1, wherein said plurality of microstructures is arranged as a plurality of microstructure arrays comprising a set of microchannels configured to carry out a set of microfluidic process steps.

3. The device of claim 1 wherein said elongate film laminate comprises a first lamina and said microstructures comprises at least one microchannel formed in said first lamina.

4. The device of claim 3 wherein said elongate lamina further comprises a second lamina having a surface affixed to said first lamina, said second lamina enclosing at least part of said microchannel.

5. The device of claim 1 wherein said elongate film laminate comprises a spacing lamina sandwiched between first and second enclosing laminae and each said microstructure comprises at least one microchannel formed as a slit through said spacing lamina, said enclosing laminae enclosing at least a part of said microstructure.

6. The device of claim 3, further comprising a flexible circuit laminate adjacent said first lamina, said flexible circuit laminate comprising at least one electrode configured to contact an electroflow medium when such medium is supplied to said microstructure.

7. A method for making a laminate device having a plurality of microstructures therein, each said microstructure being configured to carry out at least one step in a microfluidic process, said method comprising the steps of forming said microstructures in a

first lamina having a first surface, providing a second lamina having a second surface, creating a plurality of openings in at least one of the first and second lamina, and apposing the first surface of the first lamina and the second surface of the second lamina to form a laminate structure, wherein each said opening is in fluid communication with one of said microstructures.

8. The method of claim 7, further comprising the step of apposing a surface of a flexible circuit laminate adjacent said first lamina, said flexible circuit laminate comprising a plurality of electrodes, wherein each said electrode is configured to contact an electroflow medium when such medium is supplied to said microstructure.

9. The method of Claim 7 wherein said forming step includes the step of embossing the first lamina to form said microstructures therein.

10. The method of Claim 9 wherein said forming step includes the step of curing the first lamina after the embossing step.

11. The method of Claim 7 further comprising the step of supplying the first lamina from a first roll and supplying the second lamina from a second roll in a continuous feed operation.

12. The method of Claim 11 further comprising the step of cutting the laminate structure to form a plurality of discrete devices each having a plurality of microstructures thereon.

13. A method for carrying out a microfluidic process, said method comprising the steps of providing a film laminate having a plurality of microstructures arranged therein, each said microstructure being configured to carry out at least one step in the microfluidic process, each said microstructure comprising a detection region, providing a detector capable of detecting a signal produced in the course of said step in said microfluidic process, causing relative movement between said film laminate and said detector to bring said detection region into the detection field of said detector.

14. A device for carrying out a microfluidic process, said device comprising

an elongate film laminate having a plurality of microstructures arranged therein, each said microstructure being configured to carry out at least one step in the microfluidic process, each said microstructure comprising a detection region,

a detector capable of detecting a signal produced in the course of said step in said microfluidic process,

means for moving said elongate film laminate or said detector in relation to each other to bring said detection region into the detection field of said detector.

15. A microstructure device for use with first and second contact probes extending from an electrode support structure in a predetermined pattern comprising a laminate structure having a first lamina of a plastic material, the first lamina having first and second spaced-apart parallel surfaces, the first lamina being provided with at least one microstructure extending in a direction parallel to the first and second parallel surfaces, the laminate structure having first and second spaced-apart wells adapted to receive a fluid and in fluid communication with the at least one microstructure, the laminate structure having a second lamina of a nonconductive material, electrical means at least partially carried by the second lamina for each of the first and second wells, each of the electrical means having an electrode portion in communication with the fluid of the respective well and a contact portion spaced apart from the respective well and not in fluid communication with the fluid of the respective well, the contact portions being arranged on the laminate structure in a pattern corresponding to the predetermined pattern of contact probes whereby the first and second contact probes can be brought into contact with the respective contact portions so as to provide a desired voltage potential to the fluid provided in the first and second wells.

16. The device of Claim 15 wherein each contact portion is accessible from the exterior of the laminate structure.

17. The device of Claim 16 wherein the second lamina has first and second spaced-apart parallel surfaces, each electrode portion being adjacent to the first surface of the second lamina and each contact portion being adjacent the second surface of the second lamina.

18. The device of Claim 17 wherein each of the first and second electrical means extends between the first and second surfaces of the second lamina so that each contact portion underlies the respective electrode portion.

19. The device of Claim 16 wherein each of the first and second electrical means includes a trace portion which electrically connects the contact portion to the electrode portion.

20. The device of Claim 19 wherein the electrode portion of each of the first and second electrical means is disposed at a bottom of a respective well.

21. The device of Claim 20 wherein the second lamina has first and second spaced-apart parallel surfaces, each electrode portion being adjacent to the first surface of the second lamina, each contact portion being adjacent to the second surface of the second lamina and each trace portion extending transversely between the first and second surfaces of the second lamina.

22. The device of Claim 16 wherein the laminate structure has first and second spaced-apart parallel surfaces, each of the first and second wells being accessible from the first surface and each of the contact portions of the first and second wells being accessible from the second surface.

23. The device of Claim 16 wherein the laminate structure has first and second spaced-apart parallel surfaces, each of the first and second wells and each of the contact portions of the first and second wells being accessible from the first surface.

24. The device of Claim 15 for use with first and second piercing contact probes wherein the second lamina is made of a material which permits the first and second piercing contact probes to penetrate the second lamina so that the first and second piercing contact probes electrically engage the contact portions of the first and second electrical means.

25. The device of Claim 16 wherein the laminate structure has a third lamina overlying the first and second wells for sealably enclosing the fluid in the first and second wells.

26. The device of Claim 15 for use with additional first and second contact probes wherein the first lamina is provided with an additional microstructure and the laminate structure has additional first and second spaced-apart wells in fluid communication with the additional microstructure, additional first and second electrical means at least partially carried by the second lamina for the additional first and second wells.

27. The device of Claim 15 for use with an additional first and second contact probes wherein the first lamina is provided with an additional microstructure, the laminate structure has additional first and second spaced-apart wells in fluid communication with the additional microstructure and the laminate structure has a third lamina of a nonconductive material disposed adjacent the second lamina, additional first and second electrical means at least partially carried by the third lamina for the additional first and second wells.

28. The device of Claim 27 wherein the second lamina overlies the first lamina and the third lamina overlies the second lamina.

29. The device of Claim 28 wherein the first and second wells and the additional first and second wells extend through the second lamina and the third lamina.

30. The device of Claim 15 wherein the first and second wells extend through the second lamina.

31. The device of Claim 30 wherein at least one of the electrode portions is annular in shape and extends around the respective well.

32. A microstructure device for use with first and second contact probes extending from an electrode support structure in a predetermined pattern comprising a laminate structure having an exterior and a first lamina of a plastic material, the first lamina having first and

second spaced-apart parallel surfaces, the first lamina being provided with first and second microstructures extending in a direction parallel to the first and second parallel surfaces, the laminate structure having first and second wells adapted to receive a fluid, the first well being in fluid communication with the first microstructure and the second well being in fluid communication with the second microstructure, the laminate structure having a second lamina and a third lamina each of a nonconductive material, first electrical means at least partially carried by the second lamina for the first well and second electrical means at least partially carried by the third lamina for the second well, each of the electrical means having an electrode portion in communication with the fluid of the respective well and a contact portion spaced apart from the respective well and not in communication with the fluid of the respective well, the contact portions being arranged on the laminate structure in a pattern corresponding to the predetermined pattern of contact probes whereby the first and contact probes can be brought into contact with the respective contact portions so as to provide a desired voltage potential to the fluid provided in the first and second wells.

33. The device of Claim 32 wherein the second lamina overlies the first lamina and the third lamina overlies the second lamina.

34. The device of Claim 33 wherein the first and second wells extend through the second lamina and the third lamina.

35. The device of Claim 34 wherein at least one of the electrode portions is annular in shape and extends around the respective well.

36. The device of Claim 33 wherein each of the first and second electrical means includes a trace portion which electrically connects the contact portion to the electrode portion, the trace portion of the second electrical means overlying the trace portion of the first electrical means and being electrically insulated from the trace portion of the first electrical means by the third lamina.

37. The device of Claim 32 wherein the laminate structure has a fourth lamina overlying the first and second wells for sealably enclosing the fluid in the first and second wells.

38. A microstructure device for use with first and second contact probes extending from an electrode support structure in a predetermined pattern comprising a laminate structure having a first lamina of a plastic material, the first lamina having first and second spaced-apart parallel surfaces and being provided with at least one microstructure extending in a direction parallel to the first and second parallel surfaces, the laminate structure having a second lamina of a nonconductive material, the second lamina having first and second spaced-apart surfaces and being provided with a plurality of spaced-apart bores extending through its first and second parallel surfaces for forming at least a portion of a plurality of wells adapted to receive a fluid and in fluid communication with the at least one microstructure, electrical means carried by the laminate structure for each of the plurality of wells, each of the electrical means having an electrode portion in communication with the fluid of the respective well and a contact portion spaced apart from the respective well and not in fluid communication with the fluid of the respective well, the contact portions being arranged on the laminate structure in a pattern corresponding to the predetermined pattern of contact probes whereby the first and second contact probes can be brought into contact with the contact portions so as to provide a desired voltage potential to the fluid provided in the plurality of second wells.

39. The device of Claim 33 wherein the first lamina is provided with an additional such microstructure and the second lamina is provided with an additional plurality of such spaced-apart bores for forming at least a portion of an additional plurality of wells in fluid communication with the additional microstructure, additional such electrical means carried by the laminate structure for each of the additional plurality of wells, the additional electrical means overlying the first-named electrical means and being electrically insulated from the first-named electrical means.

40. The device of Claim 39 wherein the laminate structure includes a third lamina of a nonconductive material disposed between the first-named and additional electrical means.

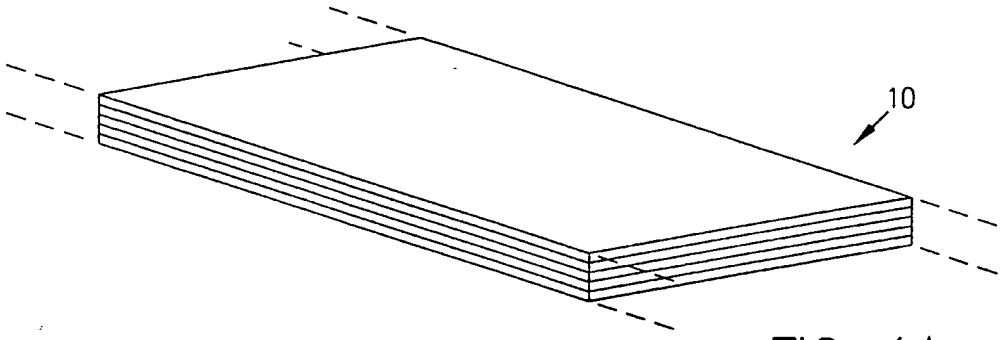

FIG. 1A

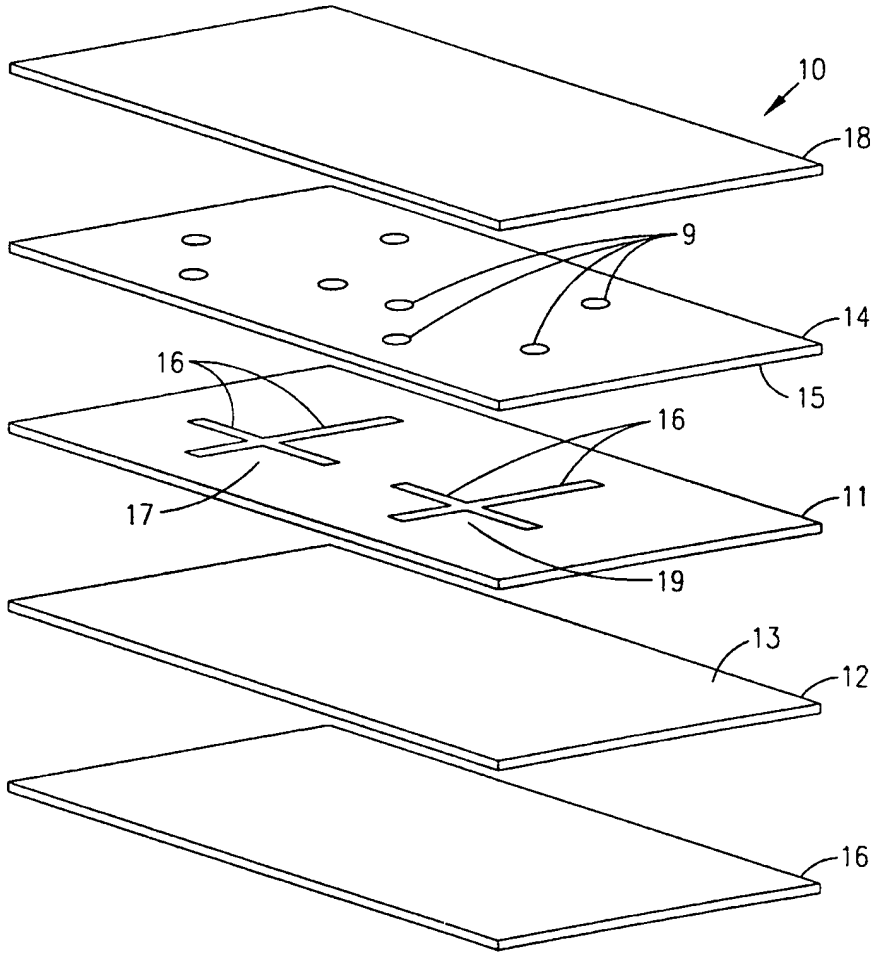

FIG. 1B

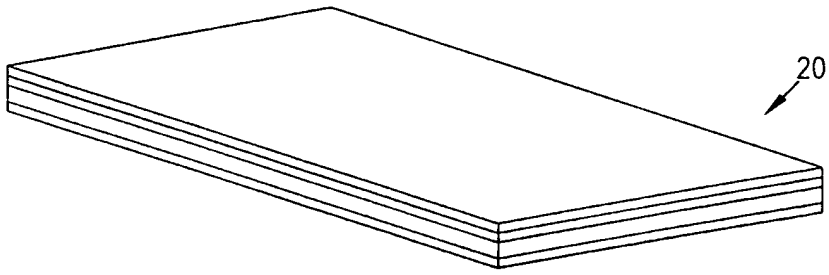

FIG. 2A

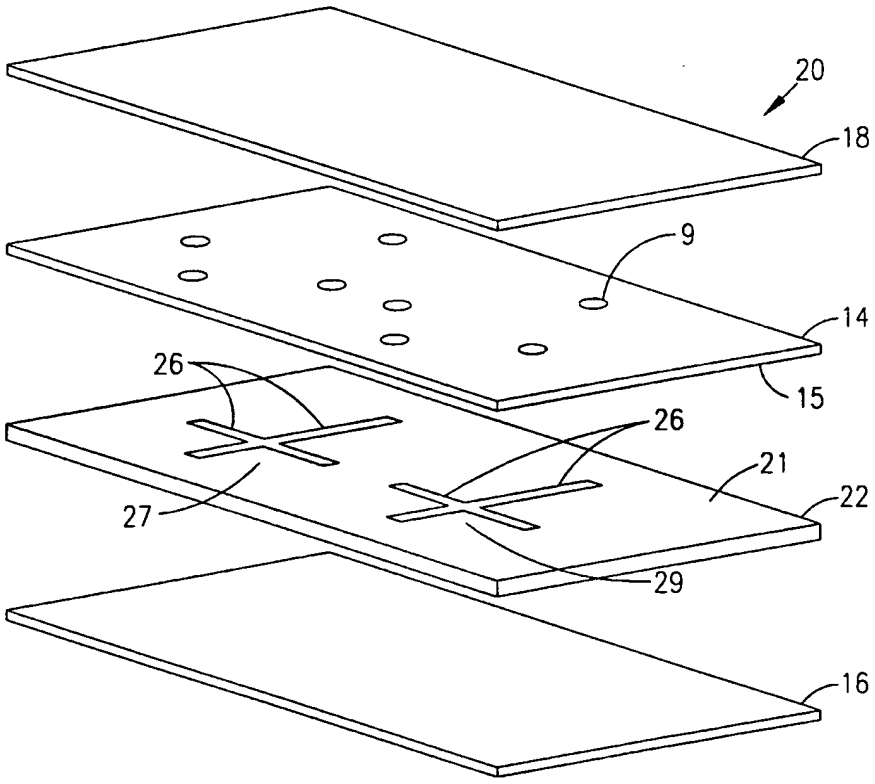

FIG. 2B

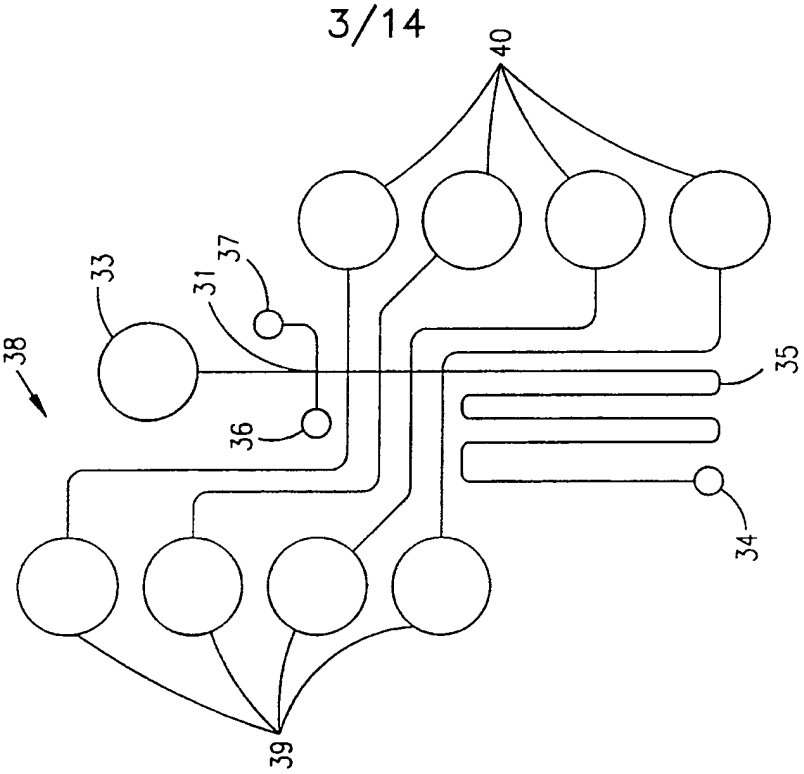

FIG. 3C

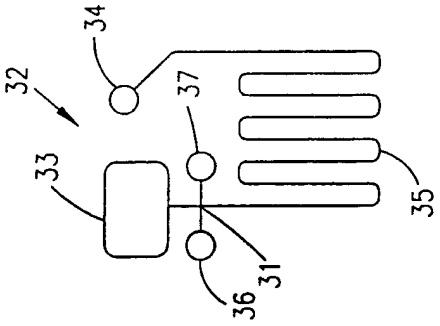

FIG. 3B

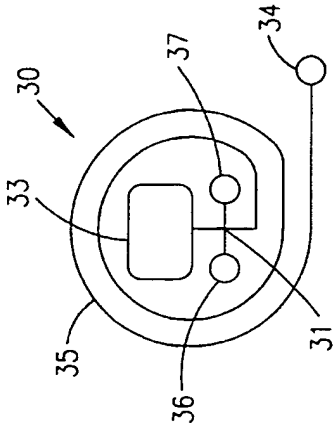

FIG. 3A

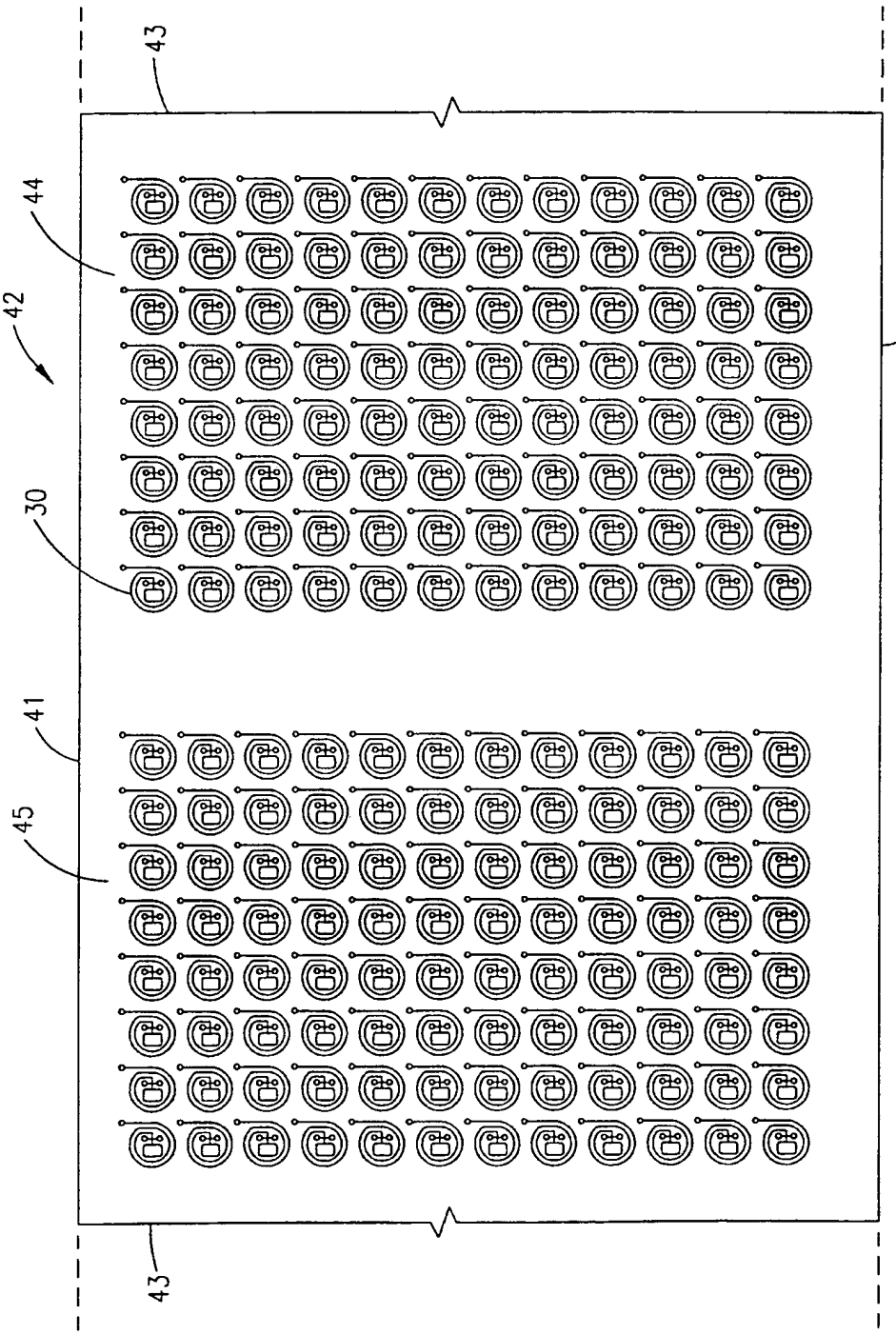

FIG. 4

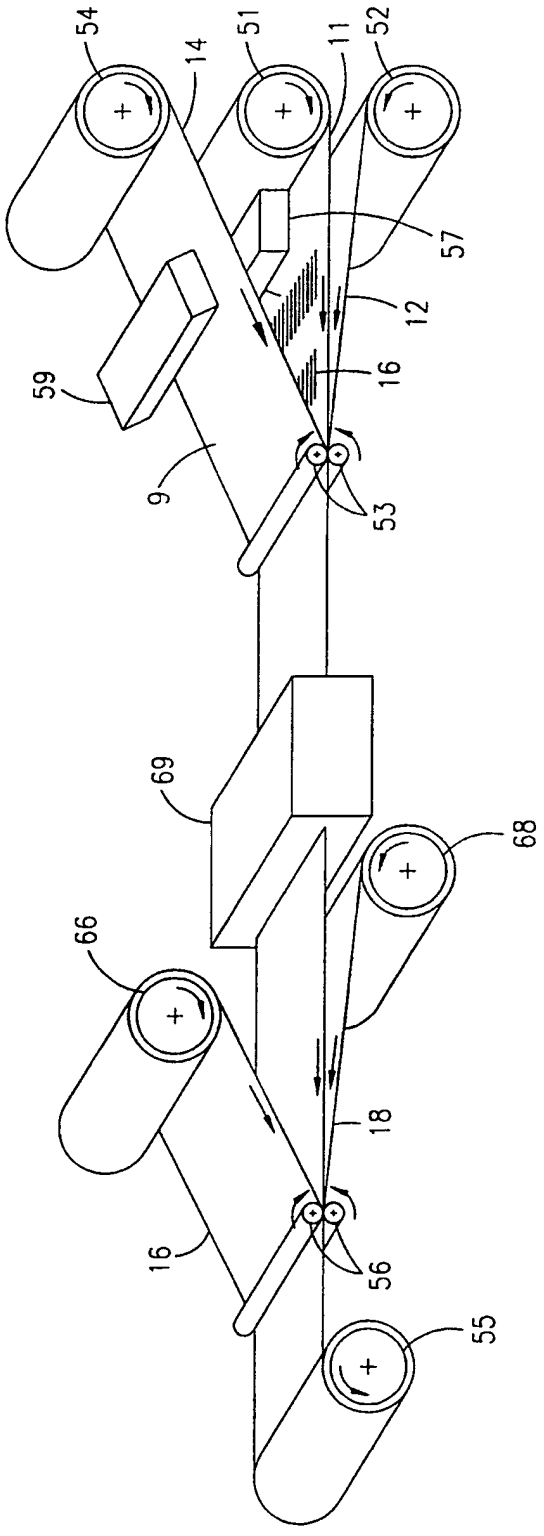

FIG. 5

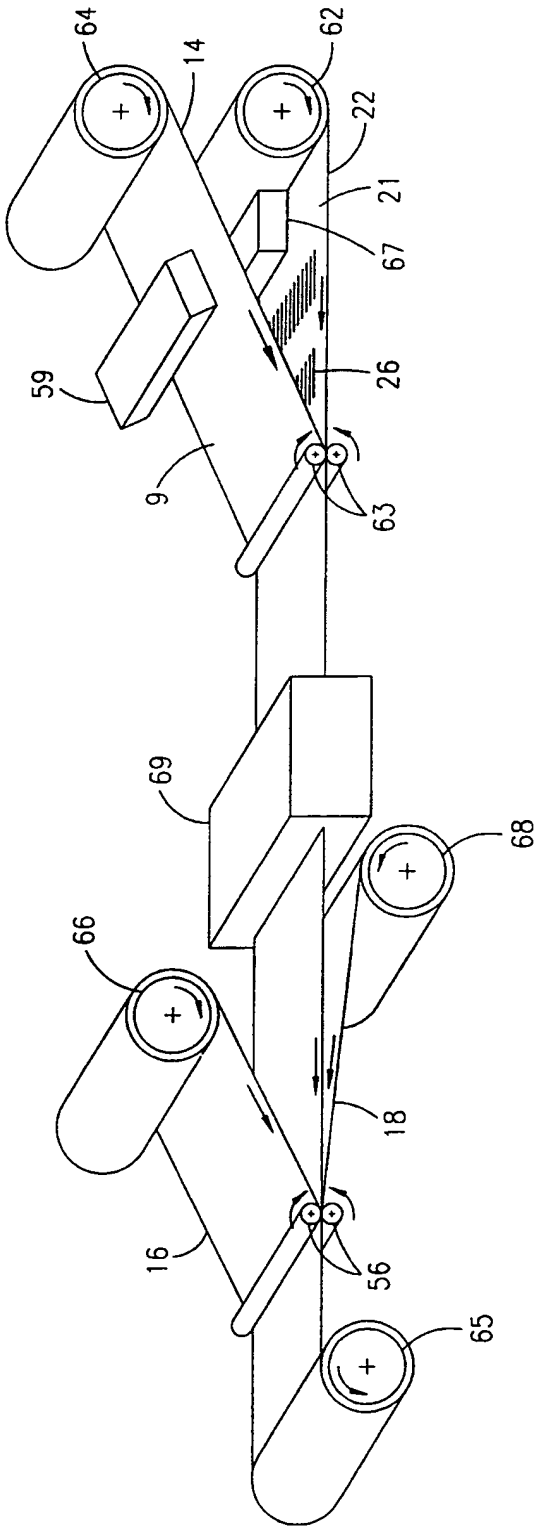

FIG. 6

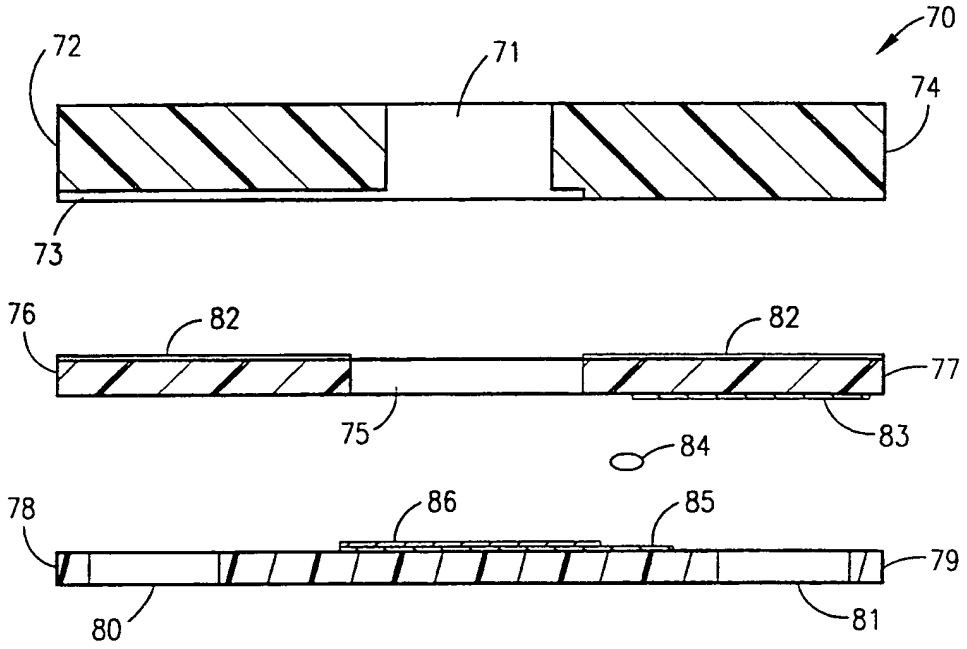
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FIG. 7a

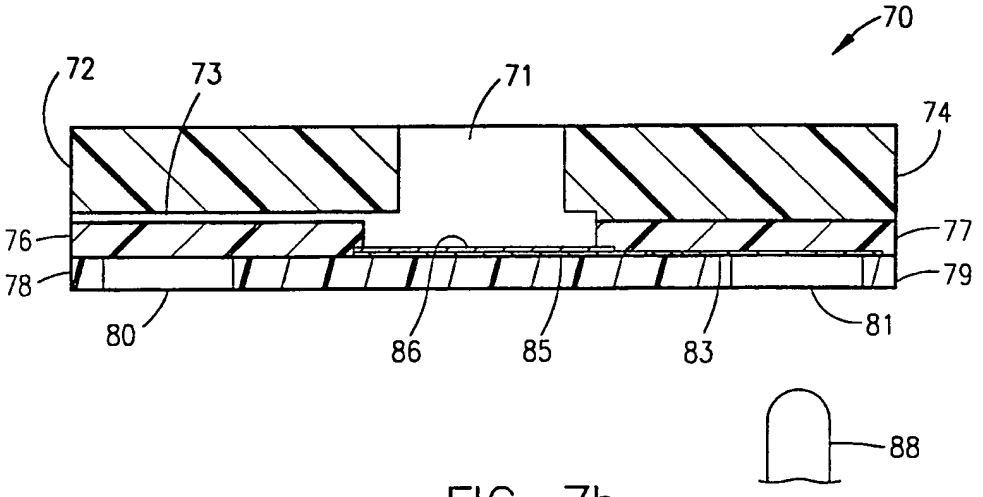
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FIG. 7b

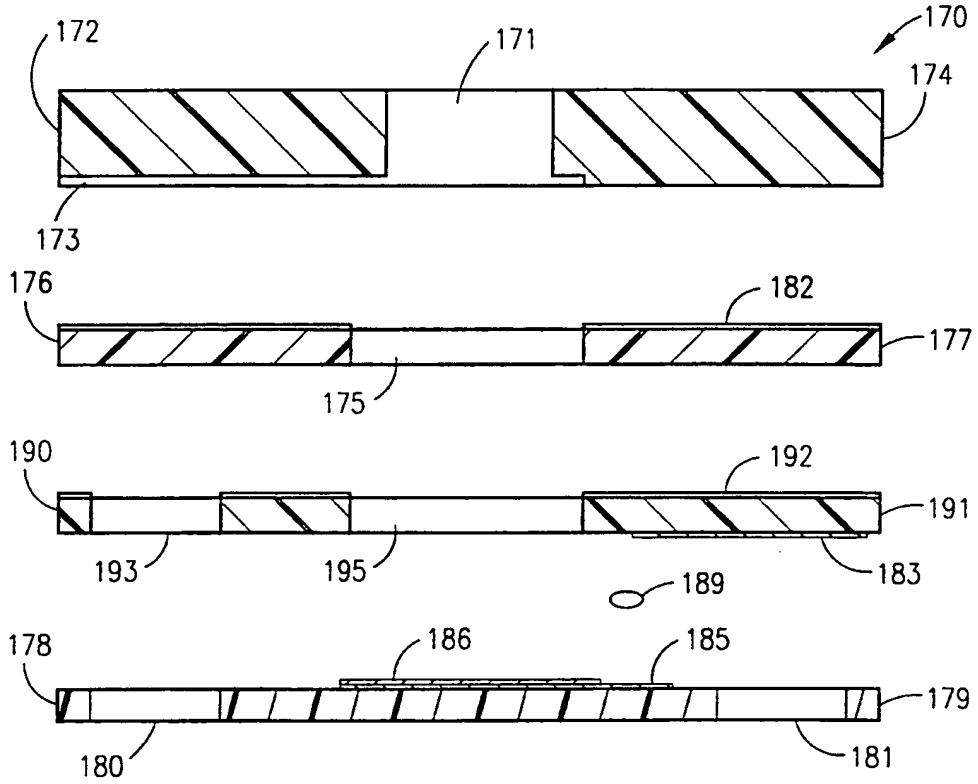
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FIG. 8a

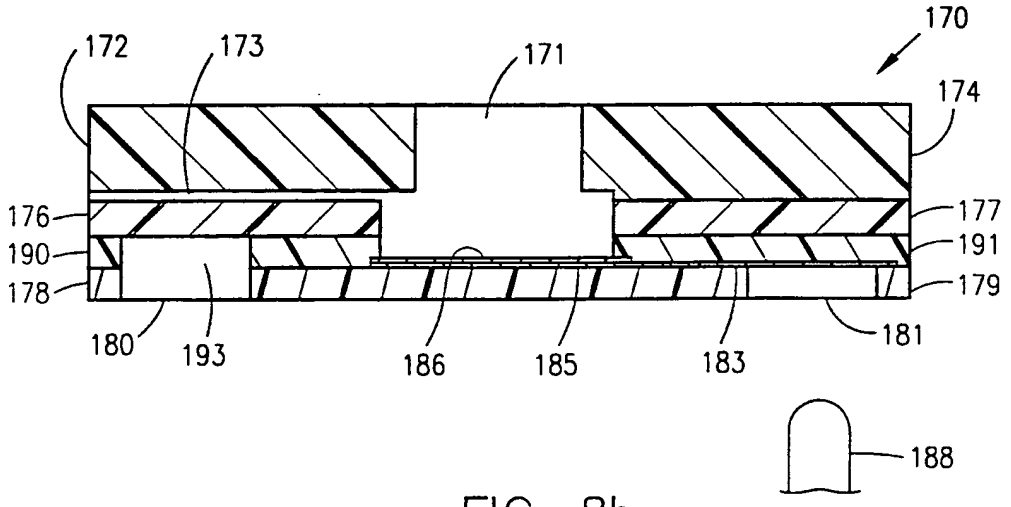
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FIG. 8b

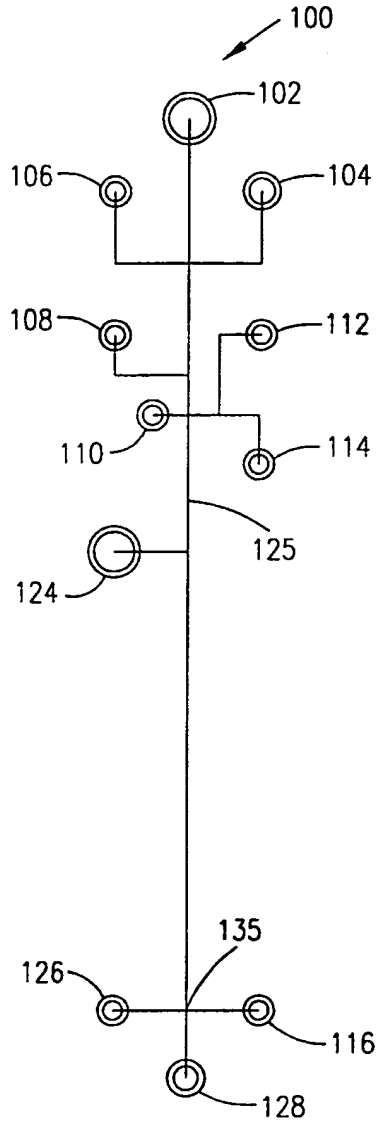
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FIG. 9

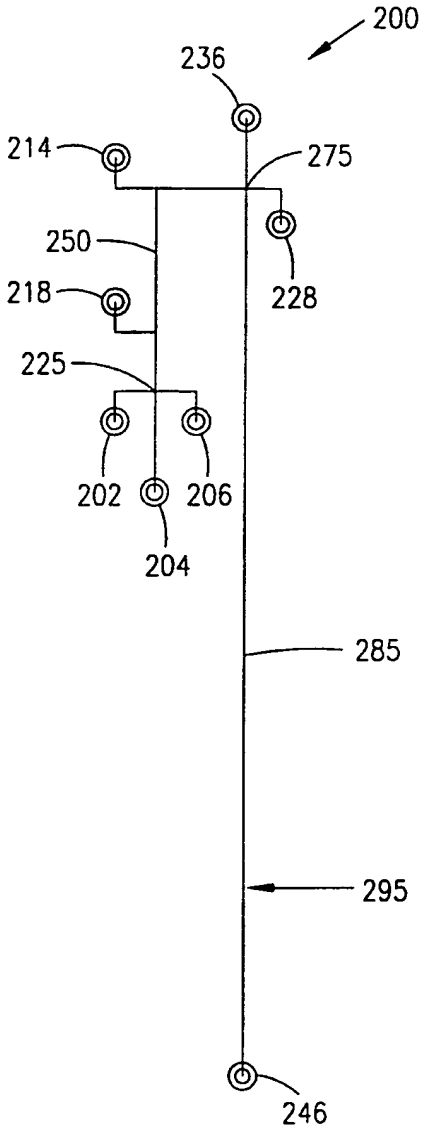
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FIG. 10

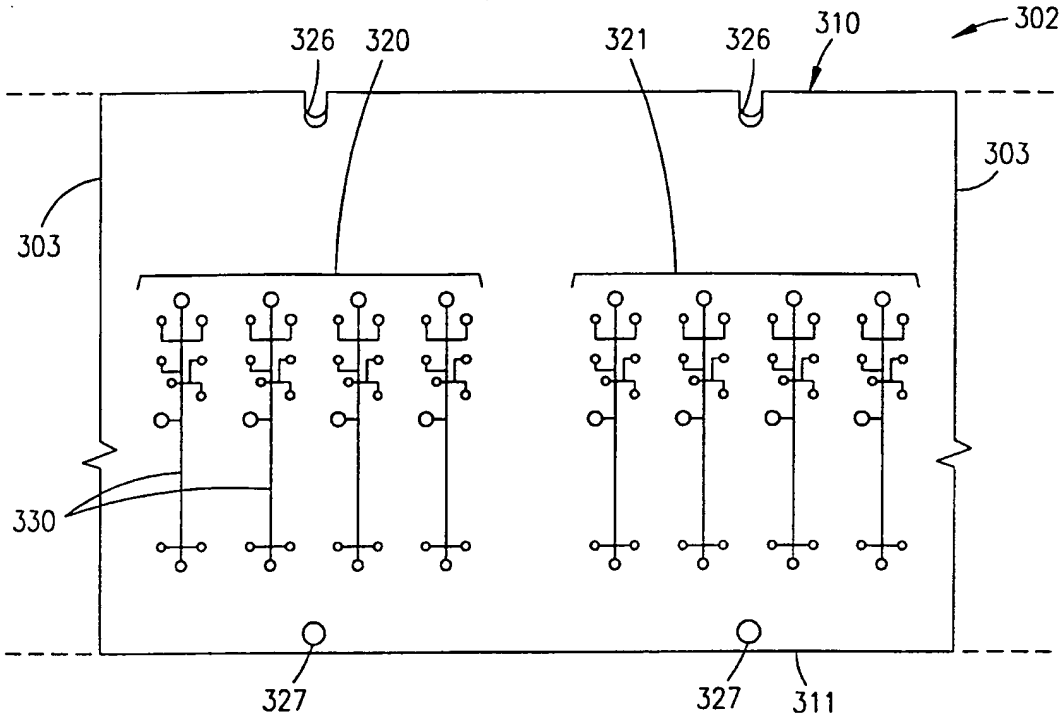


FIG. 11a

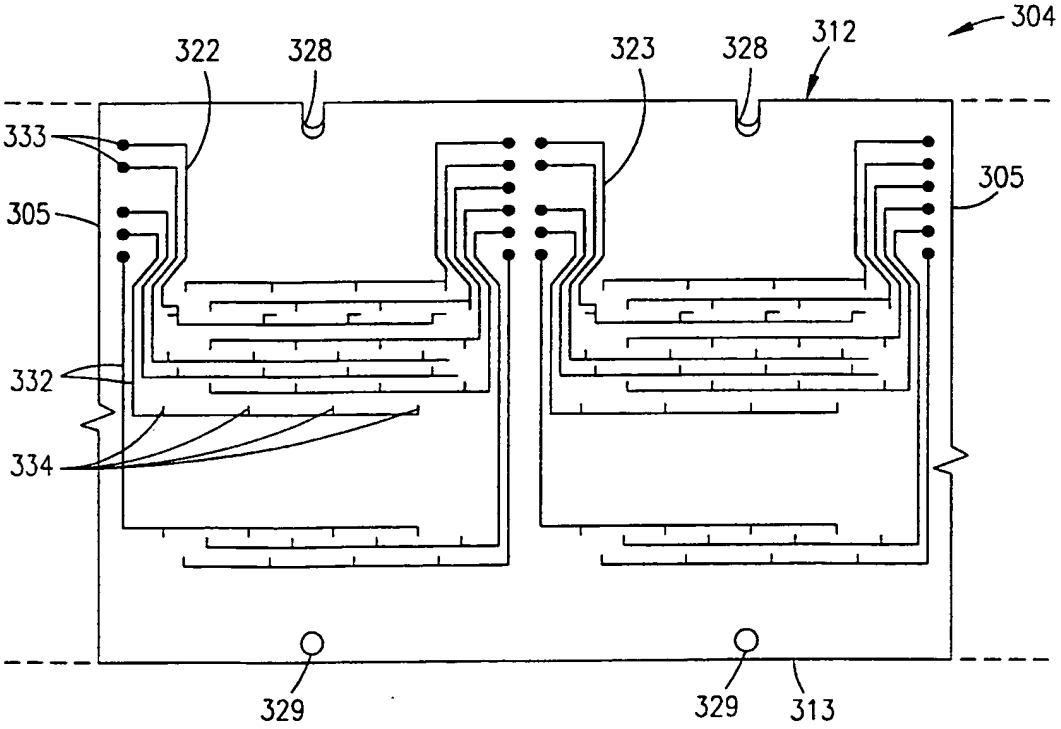


FIG. 11b

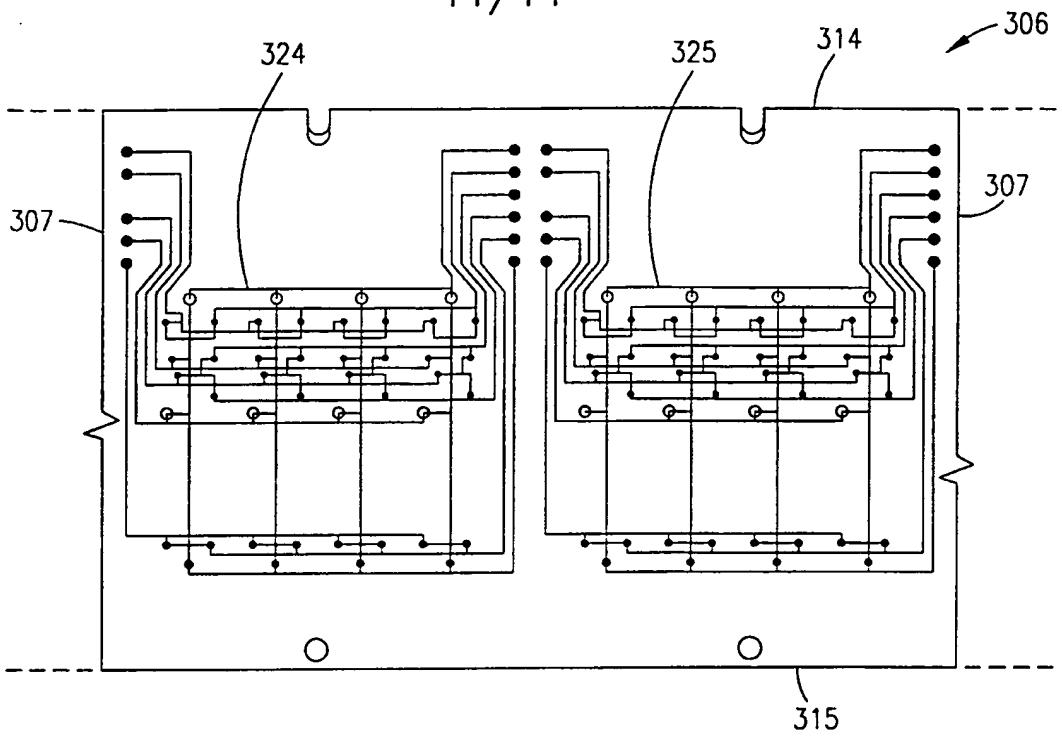


FIG. 11c

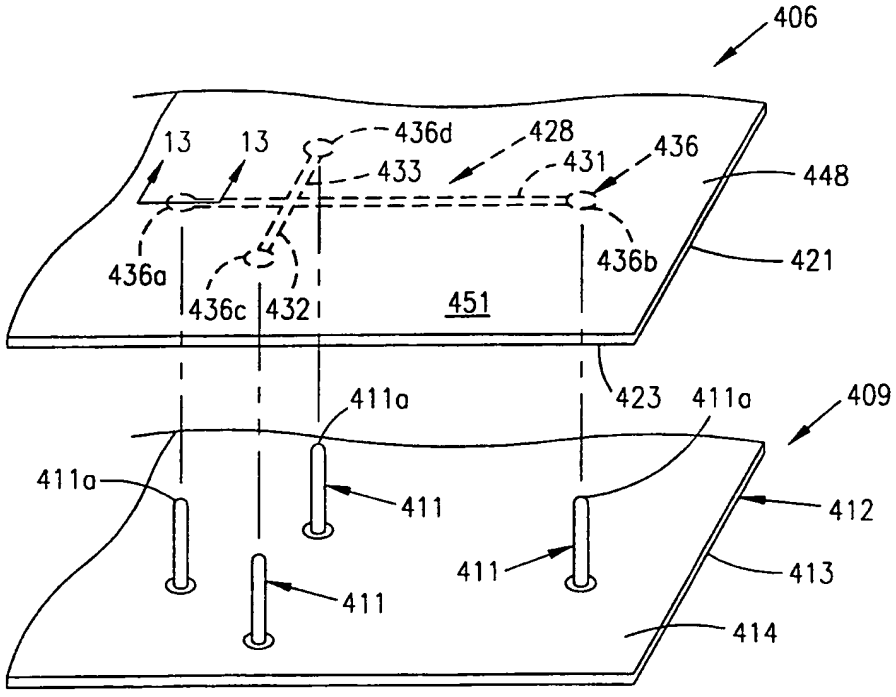


FIG. 12

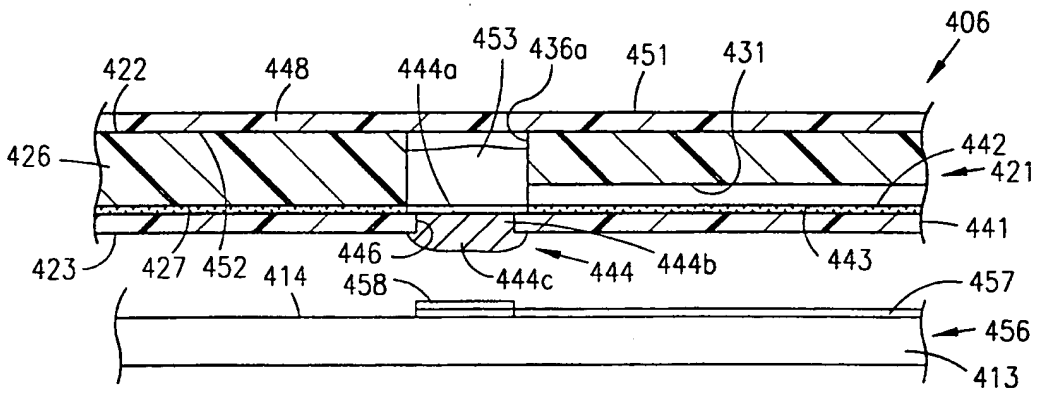


FIG. 13

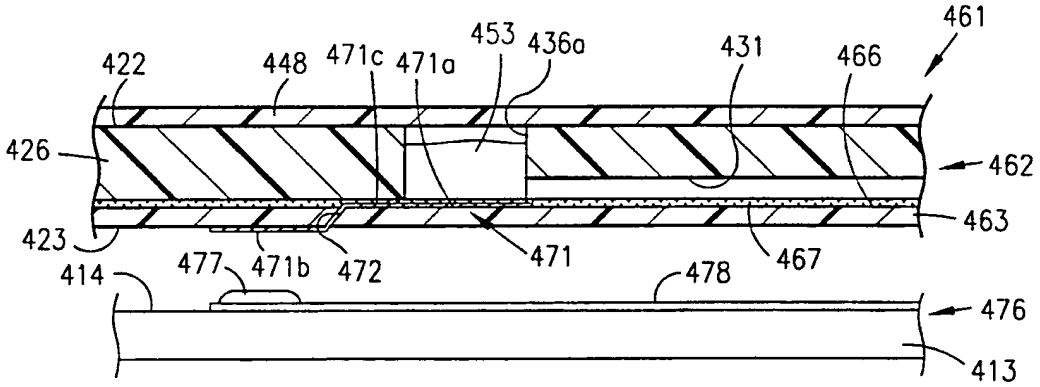


FIG. 14

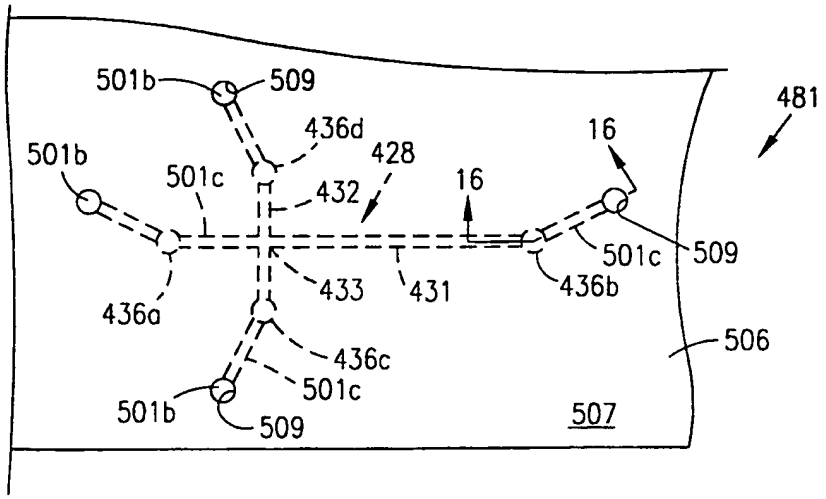


FIG. 15

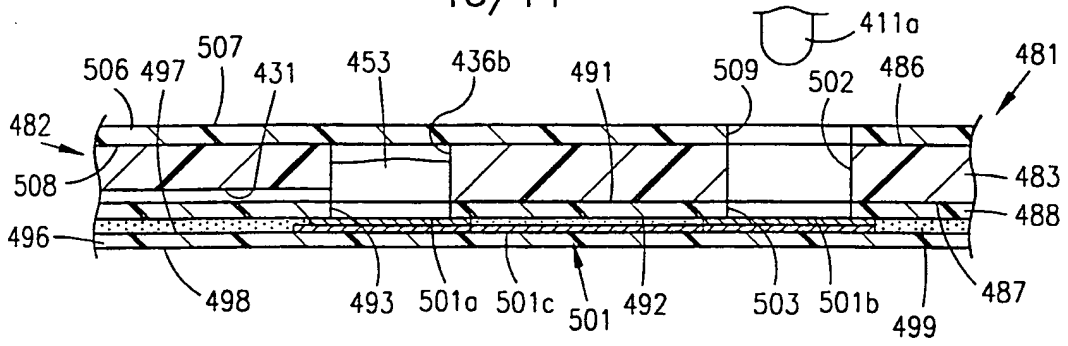


FIG. 16

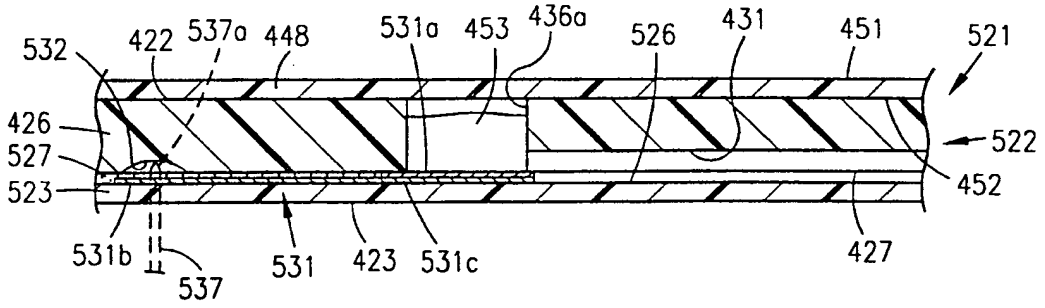


FIG. 17

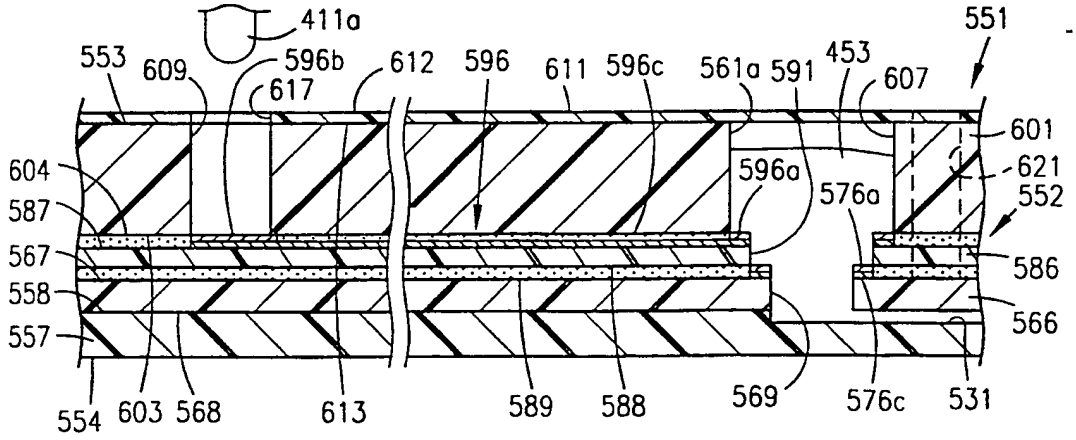
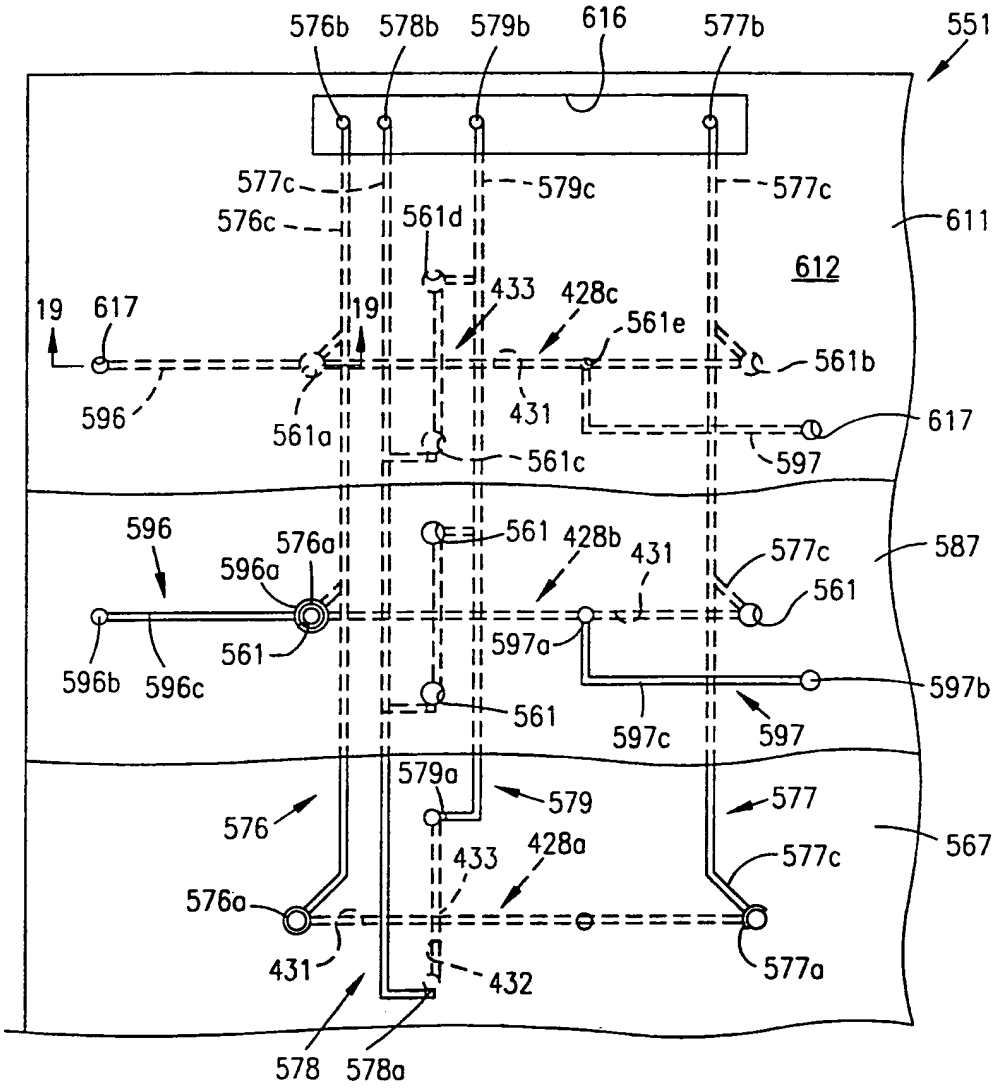


FIG. 19

14/14


576b
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578a

FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21869

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 25/22
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/58, 66, 82.01, 82.02, 100, 102; 204/601, 603; 156/145, 150, 184, 228, 242; 436/44, 149, 150

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,658,413 A (KALTENBACH ET AL) 19 August 1997 (19.08.97), column 7, lines 14-17, column 26, lines 49-67, and figures 1a and 6.	1-6
X -- Y	US 4,952,266 A (TSURUTA ET AL) 28 August 1990 (28.08.90), column 2, line 32 - column 3, line 27 and figures 4-5.	1-12 ------ 13-40
Y	US 5,030,418 A (MIYATA) 09 July 1991 (09.07.91), column 11, lines 1-27.	13-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 DECEMBER 1998	Date of mailing of the international search report 26 JAN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JAN M. LUDLOW Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21869

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/58, 66, 82.01, 82.02, 100, 102; 204/601, 603; 156/145, 150, 184, 228, 242; 436/44, 149, 150

Nucleic Acids Research, 1999, Vol. 27, No. 2 649–655

Immobilization of acrylamide-modified oligonucleotides by co-polymerization

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ABSTRACT

A flexible chemistry for solid phase attachment of oligonucleotides is described. Oligonucleotides bearing 5′-terminal acrylamide modifications efficiently co-polymerize with acrylamide monomers to form thermally stable DNA-containing polyacrylamide co-polymers. Co-polymerization attachment is specific for the terminal acrylamide group. Stable probe-containing layers are easily fabricated on supports bearing exposed acrylic groups, including plastic microtiter plates and silanized glass. Attachment can be accomplished using standard polyacrylamide gel recipes and polymerization techniques. Supports having a high surface density of hybridizable oligonucleotide (~200 fmol/mm^2) can be produced.

INTRODUCTION

Solid phase nucleic acid hybridizations are widely used in the life sciences and diagnostics. Despite the general utility of this procedure, there is little consensus on the best chemical approach for attaching nucleic acid probes to supports. A great number of attachment methods have been published, which vary widely in chemical mechanism, ease of use, probe surface density and attachment stability (1–27).

Among the most promising solid phase attachment methods developed over the last decade are those that utilize polyacrylamide supports (5,7,15,25–27). The chief advantages of these supports are high probe capacity, low non-specific binding levels and relatively high thermal stability. Moreover, it is relatively easy to manipulate probe density for normalizing hybridization properties of a probe array (7). Despite these advantages, the DNA attachment methods cited above are inconvenient in that they require activation of the gels, probes or both with labile reactive chemicals.

During development of a solid phase PCR process (E.S.Abrams *et al.*, submitted for publication), we developed a primer attachment method based on the co-polymerization of acrylamide-modified oligonucleotides into a polyacrylamide co-polymer. The method has significant advantages in stability and convenience over previous methods. First, it can be performed with standard, widely used gel polymerization techniques. Second, it provides surfaces with very high probe density. Third, the attachment is extremely stable and can withstand PCR cycling conditions. These features, along with the low non-specific nucleic acid binding of polyacrylamide supports, make the method useful for many applications and accessible to a wide range of molecular biologists and chemists.

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides containing 5′-terminal acrylamide groups were obtained commercially from Research Genetics (Huntsville, AL) or Operon Technologies (Alameda, CA). The 5′-terminal acrylamide groups were added during automated synthesis using a commercially available acrylamide phosphoramidite (Acrydite™; Mosaic Technologies, Boston, MA). All other oligonucleotides, including 5′-fluorescein-labeled, 5′-amine-modified and unmodified oligonucleotides were obtained from Ransom Hill Bioscience (Ramona, CA). Lyophilized oligonucleotides were dissolved in TE buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA) and stored frozen at –20°C. Concentrations were determined from absorbance at 260 nm (assuming 33 μg/ml oligonucleotide per 1 OD unit). All concentrations refer to oligonucleotide strands.

Electrophoresis assay of immobilization efficiency

Glass plates for a vertical polyacrylamide minigel (10 × 10 cm, 0.75 mm spacers) were assembled and the sandwich was filled approximately half way with 20% polyacrylamide (19:1; Bio-Rad), 1× TBE (90 mM Tris–borate buffer, pH 8.3, 2 mM EDTA). Polymerization was initiated by inclusion of 10% aqueous ammonium persulfate (APS) and TEMED at 1/100th and 1/1000th gel vol, respectively. After polymerization, three additional spacers were inserted vertically into the top of the plate sandwich so that they contacted the top of the gel layer. The spacers were spaced to create four laterally arranged compartments of approximately equal size on top of the gel layer. Four different 300 μl aliquots of gel solution (20% polyacrylamide, 1× TBE, 4 μl 10% APS and 4 μl 10% TEMED) were polymerized in these compartments, each aliquot containing a different oligonucleotide at a final concentration of 3 μM. Two different sequences were used for the four oligonucleotides [oligo 1, d(TTT TTT TTT ACG

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CAG CGA CGA GCA CGA GAG); oligo 2, d(TTT TTT TTT GAC TGC TGG CGG AAA ATG AGA AAA)]. Each sequence was synthesized in two versions: one with a 5′-terminal primary amine and one with a 5′-terminal acrylamide group. After polymerization of the four oligo-containing compartments, the three spacers separating the compartments were removed and the remaining space in the plate sandwich was filled with a 20% gel layer. This composite gel was then assembled in a minigel apparatus containing 1× TBE and subjected to electrophoresis at 100–150 V for ~45 min. After electrophoresis, the gel was stained with ethidium bromide and photographed using a CCD video camera with UV transillumination.

Preparation of optical fiber supports

The polished ends of silica optical fibers (1 mm diameter) were cleaned by soaking in 10% aqueous nitric acid for 2 h. The fibers were rinsed with water and acetone and then air dried. The fiber tips were then soaked in 10% 3-methacryloxypropyltrimethoxysilane (Bind Silane; Pharmacia Biotech) in acetone (v/v) for 1 h. The tips were washed in acetone and air dried.

Photochemical attachment of 5′-acrylamide oligonucleotides to optical fibers

To attach primers the silanized tips were immersed in solutions of 8% (w/v) acrylamide (17:1 w/w acrylamide:bis-acrylamide in 0.1 M phosphate buffer, pH 6.8) containing 1 µM capture probe 13B, d(TT TTT TTT TCG GGA TCC CAG GCC CGG GAA CGT ATT CAC), with a 5′-terminal acrylamide group (M samples) or 1 µM 13B probe with a 5′-terminal phosphate group (U samples). Riboflavin was added to a final concentration of 0.0006% w/v and light from a 100 W halogen lamp was passed through the distal end of the fiber for 5 min. The photopolymerized fiber tips were immersed in an agarose minigel box containing TE buffer and subjected to electrophoresis for 30 min at 100 V/cm to remove non-immobilized oligonucleotide.

Measurement of attachment stability under PCR conditions

Polyacrylamide beads containing 5′-acrylamide-modified and unmodified 13B capture probes (sequence above) were prepared by pipetting 25 µl aliquots of gel solution into microtiter plates filled with mineral oil. Gel solutions contained 20% acrylamide (19:1 w/w acrylamide:bis-acrylamide), 1× TBE, 0.5% w/v APS, 0.5% v/v TEMED and 10 µM oligonucleotide. Following polymerization, beads were washed in TE buffer and subjected to electrophoresis as described above to remove unpolymerized oligonucleotides. Beads were immersed in PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$) and thermocycled as indicated using a cycle of 94°C for 55 s, 55°C for 25 s and 72°C for 1 min. Following cycling, beads were subjected to electrophoresis again to remove any unbound oligonucleotides and hybridized with a complementary ^{32}P-labeled oligonucleotide target.

Measurement of probe density on optical fibers and beads

Probe density was determined by hybridizing complementary ^{32}P-labeled oligonucleotide, d(GTG AAT ACG TTC CCG GGC CT), to bead or fiber optic hybridization supports. The probe was labeled with terminal transferase (Amersham/Pharmacia Biotech) and [α-^{32}P]dCTP at 3000 Ci/mmol (NEN Life Sciences). Labeled oligonucleotides were separated from unincorporated nucleotides by silica adsorption (QiaAmp; Qiagen) followed by three cycles of centrifugal ultrafiltration on Microcon 3 (Amicon). Radioactivity was determined by Cerenkov counting in a liquid scintillation counter (Beckman LS-3801). Hybridizations were carried out for 30–60 min at room temperature using 200 µl reactions containing 0.1 µM ^{32}P-labeled target, TE buffer with 0.2 M NaCl and 0.5% SDS (HYB buffer). During hybridizations with bead supports, reactions were mixed gently on a rotating wheel. Following hybridization, supports were washed with three changes (~1 ml/change for fiber tips, ~0.5 ml/change for the beads) of HYB buffer and the amount of hybridized probe was determined by Cerenkov counting.

Microtiter plate hybridization

Gel solutions containing 0.5× TBE, 20% acrylamide (19:1 w/w acrylamide:bis-acrylamide), 0.07% w/v APS, 0.2% v/v TEMED, 10–50 µM oligonucleotide capture probe d(GAC TGC TGG CGG AAA ATG AGA AAA) modified with a 5′-acrylamide group were prepared. Gel solutions were pipetted into the wells of a dry untreated polystyrene microtiter plate (Nunc Polysorb, U-shaped well, 50 µl/well) and allowed to polymerize for 30 min at room temperature. Following polymerization, the wells were washed extensively with 0.5× TBE, soaked overnight at 4°C in 0.5× TBE and washed again immediately before hybridization. Hybridization was carried out at room temperature for 20 min using 1 µM complementary, d(TTC TCA TTT TCC GCC AGC AG), or non-complementary, d(TGA GGC TTG CTG TTA TGG TAC), 5′-fluorescein-modified target oligonucleotide in TE with 0.32 M NaCl. Following hybridization, wells were washed six times with 200 µl 0.5× TBE and fluorescence remaining in the wells was measured using a Molecular Dynamics Fluorimager.

Preparation of acrylamide arrays on slides

Gel solutions were prepared containing 75% glycerol, 10% total acrylamide (29:1 w/w acrylamide:bis-acrylamide), 5 µM 5′-acrylamide oligonucleotide, 0.125% w/v APS and 0.125% v/v TEMED. Aliquots of gel solution (0.2 µl) were manually pipetted onto silanized slides (acrylic silane-treated slides; CEL Associates, Houston, TX). Spotted slides were placed in a humid nitrogen atmosphere at room temperature for 5 min to allow polymerization. Polymerized slide arrays were subjected to electrophoresis in an agarose minigel box (50 mM Tris–acetate, pH 7.8, 2 mM EDTA, 20 V/cm, 20 min) to remove non-immobilized probe. Slides were rinsed in TE buffer or water and dried with a stream of nitrogen. In some cases, slide arrays were stored in TE buffer. Slides stored wet (immersed in TE) or dry (ambient temperature and humidity) showed similar performance (data not shown). The 5′-acrylamide capture probes used were: probe 1, d(CAG AAT CGT TAG TTG ATG GCG A); probe 2, d(AAT CCA AAA CGG CAG AAG); probe 3, d(GTT GCC CGT CTC GCT GGT GAA A). The capture probes were attached to the 5′-acrylamide group by an 18 atom polyethylene glycol spacer (Spacer 18 phosphoramidite, catalog no. 10-1918-90; Glen Research, Sterling, VA).

Generation of fluorescent asymmetric PCR product

A primary symmetric PCR amplification was carried out using φX174 virion target DNA using primers d(GAC TGC TGG CGG

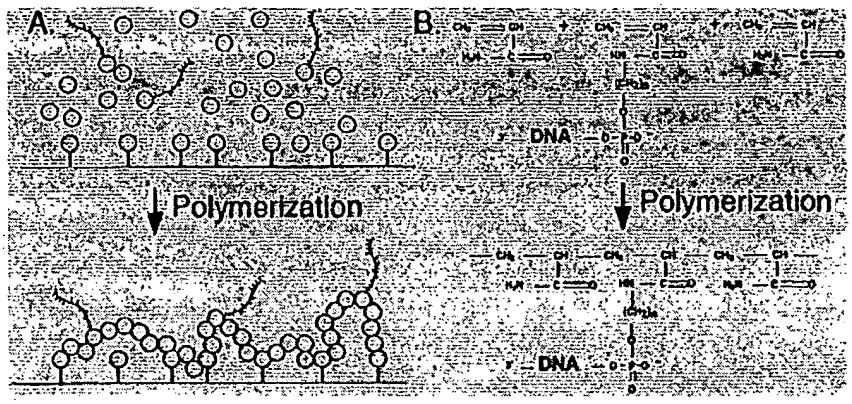

Figure 1. Co-polymerization attachment concept. (A) The circles represent polymerizable functional groups. In this paper, acrylamide and acrylamide derivatives were used. The horizontal line indicates a support that has exposed polymerizable surface groups. Oligonucleotides with 5′-terminal acrylamide groups are mixed with acrylamide monomer and bis-acrylamide and the mixture is placed in contact with the surface. After polymerization, the oligonucleotides are covalently incorporated into a polyacrylamide co-polymer which is attached to the support at multiple sites. (B) The structure of the polymer linkage between the DNA oligonucleotide and the polyacrylamide backbone is shown schematically. The acrylamide group on the 5′-end of the oligonucleotide is added during automated synthesis using an acrylamide phosphoramidite. Polymerization is catalyzed using standard chemical or photochemical methods.

AAA ATG AGA AAA) and d(ACG CAG CGA CGA GCA CGA GAG CGG TCA GTA G). The reaction contained target (10^7 copies), 1 μM each primer, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM each dNTP and 2.5 U Taq polymerase (Promega) in a 50 μl volume. Thirty cycles of amplification were performed using steps of 94°C for 20 s, 60°C for 20 s and 72°C for 40 s. A 2.5 μl aliquot of the symmetric amplication product was used as target for a 50 μl asymmetric PCR reaction using the same reaction conditions and cycling conditions except that a single 5′-fluorescein primer was used [d(GAC TGC TGG CGG AAA ATG AG)]. The asymmetric PCR product was used in slide hybridization experiments without purification.

Slide array hybridization

Hybridization reactions were performed using a 100 μl plastic chamber attached to the slide by a rectangular adhesive spacer (Frame-Seal; MJ Research, Watertown, MA). Hybridization mixtures (100 μl) contained 50 μl asymmetric PCR product in slide hybridization buffer (50 mM Tris–HCl, pH 8.0, 50 mM sodium phosphate buffer, pH 6.0, 5 mM EDTA, 0.01% w/v SDS, 200 mM NaCl). Hybridization was carried out overnight at room temperature. After hybridization, the hybridization chamber was removed and the slide was washed in 200 ml slide hybridization buffer for 1 h at room temperature with vigorous stirring. Washed slides were covered with slide hybridization buffer and scanned to detect fluorescein-tagged target using a Molecular Dynamics Fluorimager.

RESULTS

The original motivation for the work described in this paper came from our efforts to develop a primer attachment chemistry of sufficient stability for use in solid phase PCR methods. In a survey of several published oligonucleotide attachment methods, we found that all provided adequate stability and primer loading for low temperature (<50°C) hybridization procedures, but that none would withstand standard PCR cycling protocols. Because of the good thermal stability of polyacrylamide-based supports demonstrated by Mirzabekov *et al.* (25), we conceived the co-polymerization attachment method shown schematically in Figure 1. Oligonucleotide capture probes, chemically modified with a polymerizable acrylamide group (circles in Fig. 1A), are mixed with acrylamide monomer (and/or other suitable monomers) and placed in contact with a support that is also chemically modified with polymerizable groups (Fig. 1A). During polymerization of the mixture, a polymer layer containing the co-polymerized DNA probes is formed on the surface. The probes are held within the co-polymer by stable carbon–carbon bonds (Fig. 1B) and the polymer layer is attached to the underlying support at many points (Fig. 1A), creating an extremely durable hybridization surface.

Oligonucleotides containing polymerizable acrylamide groups at their 5′-terminal positions were synthesized using standard automated β-cyanoethyl phosphoramidite chemistry with a commercially available acrylamide phosphoramidite (Acrydite™ phosphoramidite; Mosaic Technologies, Boston, MA). To illustrate the specificity of co-polymerization attachment, oligonucleotides with 5′-terminal acrylamide groups were co-polymerized into block-shaped regions within a 20% polyacrylamide (non-denaturing) minigel. As controls, identical oligonucleotides containing 5′-terminal amines instead of acrylamide groups were cast into adjacent regions within the gel. Following polymerization, the samples were subjected to electrophoresis for sufficient time to move non-immobilized oligonucleotides out of the blocks. An image of the ethidium bromide stained gel is shown in Figure 2. Quantification of the fluorescent signals demonstrates that ~83–84% of the oligonucleotides with 5′-acrylamide modification were firmly attached within the blocks. In contrast, only 6–9% of the 5′-amino oligonucleotides were immobilized within the blocks. These results demonstrate that immobilization of 5′-acrylamide oligonucleotides by co-polymerization is dependent on the presence of the acrylamide group and that non-specific attachment by other groups occurs at a 10-fold lower level.

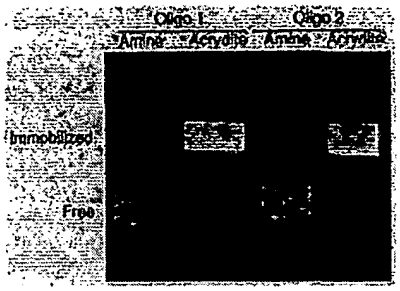

Figure 2. Specificity of co-polymerization attachment. Two pairs of oligonucleotides (lanes marked Oligo 1 and Oligo 2) were synthesized. Each pair of oligonucleotides had the same base sequence, but one had a 5′-terminal primary amine modification (Amine lanes) and the other had 5′-terminal acrylamide modification (Acrydite lanes). The four oligonucleotides were co-polymerized into gel blocks within a non-denaturing polyacrylamide electrophoresis gel as described in Materials and Methods. The gel was briefly subjected to electrophoresis, stained with ethidium bromide and imaged using a Molecular Dynamics Fluorimager.

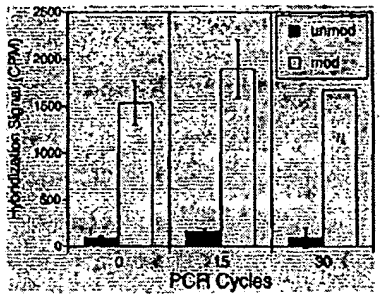

Figure 3. Stability of capture probes during PCR thermocycling. Oligonucleotides modified with 5′-acrylamide groups (mod, open bars) and unmodified oligonucleotides of identical sequence (unmod, solid bars) were co-polymerized separately into polyacrylamide beads (Materials and Methods). Beads of each type were thermocycled for the indicated number of cycles, electrophoretically washed to remove non-immobilized probe and hybridized to a complementary ^{32}P-labeled oligonucleotide target. The amount of hybridized target was determined by Cerenkov counting. The values represent the mean ± SD from duplicate measurements.

One of the original motivations for developing the co-polymerization method was the potential for achieving thermally stable attachment. This is expected since the connections between the polymer and oligonucleotide are stable carbon–carbon or carbon–nitrogen bonds. In addition, each oligonucleotide is attached to the support at many points through a crosslinked polymer network (Fig. 1). To test the thermal stability of the gel attachment, polyacrylamide beads (25 µl volume) containing 5′-acrylamide oligonucleotides or unmodified oligonucleotides of identical sequence were prepared (Materials and Methods) and subjected to a standard program of thermocycling suitable for PCR. Following thermocycling, the beads were subjected to electrophoresis to remove non-immobilized primers and then hybridized with a complementary ^{32}P-end-labeled oligonucleotide to assess primer loss during thermocycling. The results of the hybridization assay, shown in Figure 3, demonstrate that no systematic primer loss can be detected. The beads polymerized with unmodified oligonucleotide showed a low background signal, indicating that non-specific co-polymerization of unmodified oligonucleotides was very low. Using the specific activity of the probe and the calculated surface area of the beads, the apparent surface density of primer was estimated to be ~40 fmol/mm^2.

Co-polymerization attachment of oligonucleotide probes to plastic surfaces, in this case microtiter plates, is shown in Figure 4. Gels (50 µl/well) containing 5′-acrylamide oligonucleotides, 5′-amine oligonucleotides or no oligonucleotides were cast in duplicate wells of a standard untreated polystyrene microtiter plate. The plates were washed overnight to remove non-immobilized capture probe and hybridized with complementary and non-complementary fluorescein-labeled oligonucleotide. After washing away non-hybridized sample, hybridized target oligonucleotides were detected using a 2-dimensional scanning imager. As seen in Figure 4, complementary labeled oligonucleotides hybridized strongly to wells containing 5′-acrylamide probes. Hybridization of complementary probe to 5′-amine capture probes was not observed. Non-specific binding of non-complementary labeled oligonucleotides to any of the wells was not observed either. By comparison with a series of fluorescein standard samples (data not shown), the well containing 50 µM 5′-acrylamide probe

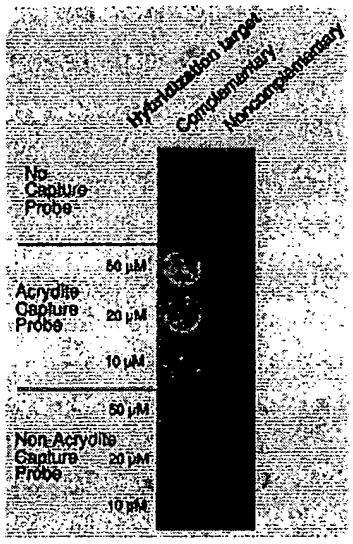

Figure 4. Immobilization of 5′-acrylamide oligonucleotides in microtiter plates. Acrylamide mixtures containing a 5′-acrylamide oligonucleotide (Acrydite Capture Probe), an unmodified oligonucleotide of identical sequence (Non-Acrydite Capture Probe) or no oligonucleotide (No Capture Probe) were polymerized in the wells of an untreated polystyrene microtiter plate. Non-immobilized probes were washed away by an overnight incubation in buffer. The wells were hybridized to complementary (left column) and non-complementary (right column) fluorescently labeled oligonucleotide targets. The fluorescent image of the plate was obtained using a Molecular Dynamics Fluorimager.

captured ~10 pmol oligonucleotide. Using the simplifying assumption that all of the hybridization occurred on the surface of the acrylamide layer, the apparent capture probe density is estimated to be 260 fmol/mm^2. The polyacrylamide layers remained securely attached to the plate, even after a 1 h incubation in hybridization buffer at 70°C.

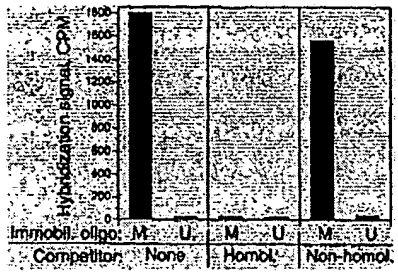

Figure 5. Hybridization to photopolymerized fiber optic supports. Polyacrylamide layers containing 5′-acrylamide capture probes were photopolymerized onto the ends of glass optical fibers as described in Materials and Methods. Fiber tips were hybridized to complementary ^{32}P-labeled oligonucleotide targets alone (left), complementary target in the presence of 100-fold excess of unlabeled complementary target (center) and complementary target in the presence of 100-fold excess of unlabeled non-complementary target (right). From the specific activity of the probe (~12 000 c.p.m./pmol), the probe density on the fibers is ~0.15 pmol, corresponding to 190 fmol probe/mm^2 area.

Numerous photochemical systems have been used to catalyze acrylamide gel polymerization. Light-directed polymerization has been developed by Walt and co-workers for fabrication of miniature multi-element chemical sensors and hybridization arrays (5,28). Photopolymerization is especially attractive for these purposes since highly focused light sources can be used to generate probe or primer arrays with small features. In addition, photopolymerization processes eliminate the need to dispense actively polymerizing monomer solutions.

To test the use of photochemical catalysts in our co-polymerization method, 5′-acrylamide oligonucleotides were photopolymerized onto the tips of glass optical fibers using riboflavin as a photoinitiator. Our procedure was adapted from Barnard and Walt (28). The fiber tips were functionalized with a polymerizable acrylic silane. To generate the photopolymerized layer, the silanized tip was immersed in acrylamide/probe solution and illuminated from the opposite end with a strong white light source. A small drop of polymerized polyacrylamide was formed on the fiber tip. Polymerized tips were washed electrophoretically and hybridized with a ^{32}P-labeled complementary target to assess capture probe density. Figure 5 shows that tips photopolymerized with 5′-acrylamide probes showed a strong hybridization signal. In contrast, tips polymerized with unmodified probes showed no hybridization signal above background. The specificity of the hybridization to the 5′-acrylamide probes is seen from the effect of unlabeled competitor hybridization targets: homologous competitor in 100-fold excess eliminated hybridization signal while non-homologous competitor reduced signal by only 15%. Estimating the surface area of the tip as a flat circle with the same diameter as the fiber, the estimated surface density of the capture probes is ~200 fmol/mm^2, in rough agreement with the value obtained from the microtiter plate experiment (Fig. 4).

The co-polymerization attachment technology is easily adapted for use in fabricating glass hybridization arrays as demonstrated in Figure 6. Acrylamide mixtures containing three different 5′-acrylamide probes were spotted in triplicate and polymerized onto the surface of a glass microscope slide. The slide had been pretreated with an acrylic silane to introduce surface co-polymerizable groups. The slide was hybridized to a fluorescein-labeled asymmetric PCR product complementary to two of the immobilized probes.

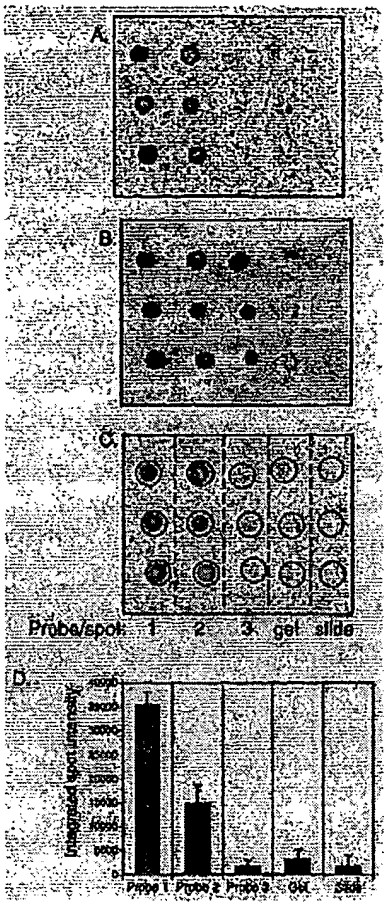

Figure 6. Hybridization of PCR products to co-polymerized 5′-acrylamide probes on glass slides. (A) Acrylamide mixtures containing three different 5′-acrylamide probes [spots 1–3 in (C)] or lacking probe [spots gel in (C)] were manually spotted onto the slide in triplicate and allowed to polymerize. The array was electrophoretically washed to remove non-immobilized probe. The slide was hybridized with a 435 base fluorescently labeled asymmetric PCR product. Probes 1 and 2 are complementary to the PCR target, but probe 3 is not. Following hybridization, the slide was washed to remove unhybridized target and scanned using a Molecular Dynamics Fluorimager. (B) To confirm the presence of the non-complementary probe 3, the hybridized array was stained with SYBR green I, which stains both single-stranded and double-stranded DNA, and rescanned. (C) The image of (A) is reproduced, showing the positions of each probe spot in the array. The regions circled were used for calculation of integrated fluorescence intensity as shown in (D). The positions marked gel contain three polyacrylamide spots without co-polymerized probe and positions marked slide are three equivalently sized areas of the bare slide. (D) The average integrated fluorescence intensity from each set of spots in the image of (A) is shown. Error bar length represents twice the SEM.

Following hybridization and washing, the slide was scanned on a fluorescence imager to reveal the distribution of hybridized target as shown in Figure 6A. That image shows high fluorescent signal on the spots containing probes 1 and 2, which are complementary to different positions within the asymmetric PCR product. Very little fluorescent signal is seen on the spots containing non-complementary probe (probe 3) or underivatized polyacrylamide gel (gel). To verify that the probe 3 spots did

contain immobilized oligonucleotide, the slide was stained with a fluorescent DNA stain (SYBR green I) and rescanned to produce the image shown in Figure 6B. Averaged hybridization and background signals from the image shown in Figure 6A are plotted in Figure 6D, using the spot definitions shown in Figure 6C. Non-specific target binding to non-complementary probe 3 is indistinguishable from the background signals of underivatized polyacrylamide gel (gel) or adjacent regions of the glass slide (slide, Fig. 6C and D). Hybridization signals from the spots containing complementary probes 1 and 2 exceeded background levels by 19- and 8-fold, respectively.

DISCUSSION

The co-polymerization attachment method described in this report has several important advantages over existing methods for DNA immobilization. It is easy to prepare co-polymerizable probes by automated DNA synthesis using an acrylamide phosphoramidite (Material and Methods). Probe immobilization can be accomplished with standard inexpensive gel polymerization techniques that are already widely used in molecular biology laboratories. Immobilization does not require highly reactive and unstable chemical crosslinking agents. The 5′-acrylamide probes co-polymerize efficiently with acrylamide and probe attachment is highly specific for the terminal acrylamide group (Fig. 2). Larger co-polymerizable probes can also be generated using PCR with 5′-acrylamide primers, since the thermocycling reaction has no effect on the terminal acrylamide groups (data not shown).

Attachment of co-polymerized probes is thermally stable. The immobilized probes are joined to the polyacrylamide layer by carbon–carbon bonds and the layer is attached to the support at multiple points (Figs 1 and 3). The results of Figure 3 have been confirmed in other experiments using 5′-acrylamide primers immobilized on glass for solid phase PCR applications (E.S.Abrams *et al.*, in preparation).

High probe densities are achievable. Inclusion of 5′-acrylamide probes at 10 μM in the polymerization mixture yields gels with apparent surface densities of ~200 fmol hybridizable probe/mm^2 (Figs 4 and 5). This value compares favorably with literature values using other attachment methods, which range from 20 to 500 fmol/mm^2 (4,8,10,12,17,29). Interestingly, in two studies using different supports, aminated polypropylene (29) and glass with a phenylenediisothiocyanate linkage (4), the optimum probe densities for hybridization were similar, ~300 and 330 fmol/mm^2, respectively. These values are very close to the apparent probe density achieved by our co-polymerization method (Figs 4 and 5). Further experiments will be required to evaluate hybridization efficiency in our system at higher probe density.

Co-polymerization of oligonucleotide probes into acrylamide-based co-polymers has been independently proposed by two other groups (30,31). Ozaki *et al.* (30) used a method very similar to ours to coat the walls of silica capillaries with oligonucleotide-containing co-polymers for affinity capillary electrophoresis applications. Muscate *et al.* (31) generated linear, uncrosslinked oligonucleotide-containing co-polymers also for affinity capillary electrophoresis applications.

Our method also resembles that of Livache *et al.* (11,32) in some aspects. Their method for DNA attachment is based on co-polymerization of pyrrole-modified oligonucleotides into a polypyrrole co-polymer. Their method differs from ours in that co-polymerization is electrochemically catalyzed and the probe-containing co-polymer is deposited onto an electrode. The polypyrrole hybridization surface had good thermal and chemical stability.

Acrylamide-modified probes can also be immobilized in standard polyacrylamide slab gels for electrophoresis, as shown in Figure 2. In other work, we have demonstrated that electrophoresis of single-stranded samples through polyacrylamide gels containing immobilized probes is an efficient and highly specific method for performing hybridization reactions (33; P.W.Hammond and T.C.Boles, in preparation).

The co-polymerization process described here should be generally useful for fabricating a wide variety of DNA-containing polymers, including microparticles, dendrimers, linear soluble polymers and polymer coatings. It should be especially useful for synthesis of self-assembling polymers that utilize specific nucleic acid base pairing interactions as an organizing principle (1,13,15,34,35). The use of DNA-containing co-polymers could significantly enhance future efforts to develop polymeric self-assembling systems, which have been primarily based on polymer–polymer and polymer–solvent interactions (36). The co-polymerization method described here should accelerate development of these materials.

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